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EFFECT OF EXTRACTION USING ION-EXCHANGE RESINS ON BATCH MIXED-ACID FERMENTATIONS

A Thesis

by

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MASTER OF SCIENCE

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ABSTRACT

Biofuels are increasingly gaining importance as an energy source. The MixAlco® process is a biomass-to-energy technology that uses mixed cultures of microorganisms to convert biomass to mixed carboxylic acids. Using a buffer, the acids are neutralized to their corresponding salts, which are recovered from the fermentation broth. Finally, the carboxylate salts are chemically converted to mixed-alcohol fuels, hydrocarbon fuels (gasoline, jet fuel, etc.), and industrial chemicals. To be a viable alternative to fossil fuels, biofuels must be economical, provide a net energy gain, and be easily produced in large quantities. Previous studies have shown that as product concentration increases, the fermenting microorganisms are increasingly inhibited, which lowers production rates and yield.

Maintaining low carboxylate salt concentration in fermentors reduces product inhibition and allows microorganisms to function efficiently and increase biomass digestion. In this study, anion-exchange resin (Amberlite IRA-67) was used to extract carboxylate salts from fermentation broth and thereby maintain near-neutral pH. Three different batch fermentations were performed using the following substrates: α-cellulose powder, shredded office paper, and lime-pretreated corn stover. It was observed that periodic extraction of carboxylate salts reduced product inhibition and thereby increased acid production in the fermentors. For all substrates, fermentors that employed an ion-exchange resin column for acid extraction had higher yield than fermentors where magnesium carbonate ($MgCO_3$) was used as a buffer to maintain pH.

The yield expressed as gram total carboxylic acid produced per gram non-acid volatile solids (NAVS) fed were 0.15 ($MgCO_3$ control) and 0.35 for α-cellulose substrate, 0.24 ($MgCO_3$ control) and 0.37 for paper substrate, and 0.20 ($MgCO_3$ control) and 0.35 for lime-pretreated corn stover. Other fermentation parameters such as conversion and selectivity also improved with periodic carboxylic acid extraction. The production of high-molecular-weight acids significantly increased for fermentors with IR extraction. This is important to the MixAlco® process because it generates high-energy products.

DEDICATION

To my parents, for their love, support and encouragement.

And to my Lord, the source of my strength.

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CHAPTER I

INTRODUCTION

Energy consumption worldwide is rapidly increasing. According to the United States Energy Information Administration (U.S. EIA) [2], almost 85% of all energy consumed in the United States of America comes from fossil fuels of which over half is consumed by the industrial and transportation sector. The U.S. EIA has projected that by 2040, 75% of total world energy consumption will still depend on fossil fuels. Because the production rate of fossil fuels is slower than its consumption rate, alternative sources of energy are needed. In addition, alternative energy will address concerns over energy security, oil prices, environmental consequences, and sustainable development [3].

In this context, biofuels are gaining increasing importance as an energy source. Use of low-ethanol blends (E5 to E25) in engines is commonly practiced. However, use of crops such as corn, soybeans, and sugar crops for fuel production conflicts with its use as food and competes with land, water, and energy resources for production [4]. In contrast, lignocellulosic biomass is abundant and in energy production ranks right after oil, coal, and natural gas. Non-food lignocellulosic biomass not only requires fewer agricultural inputs than annual crops, but also produces higher net energy [5]. Furthermore, there is no net increase in atmospheric carbon dioxide (CO_2) by combusting liquid biofuel produced from biomass because the same amount of CO_2 was removed from the atmosphere during biomass growth [6]. Although biofuels have multiple environmental benefits, to be a viable alternative to fossil fuels, they must be

economical, provide a net energy gain, and be easily produced in large quantities without affecting food supplies. The MixAlco® process (Figure 1-1), developed in the laboratory of Dr. Mark Holtzapple at Texas A&M University, is a promising biomass-to-energy technology that converts biomass to mixed-alcohol fuels, hydrocarbon fuels (gasoline, jet fuel, etc.), and industrial chemicals.

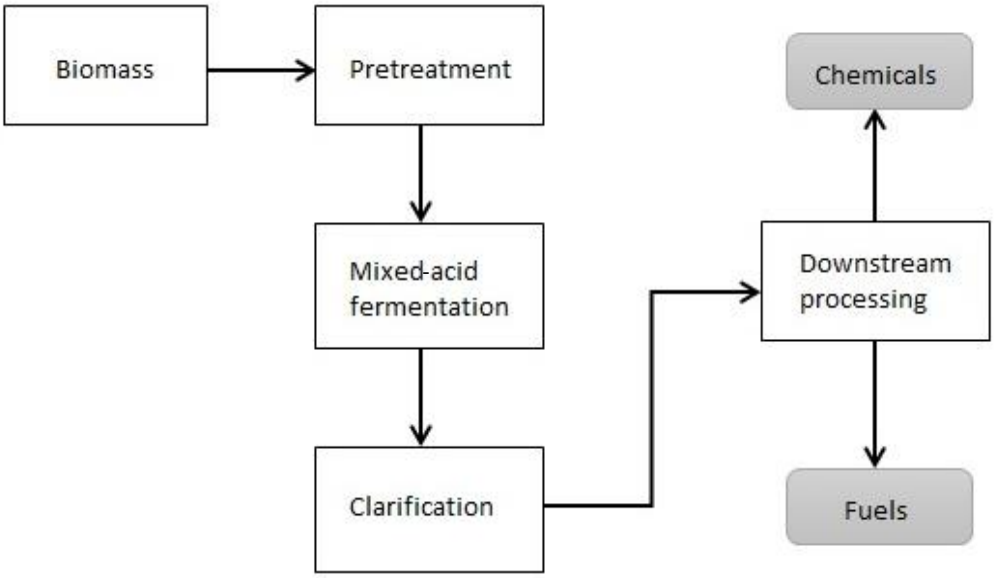


Figure 1-1 Diagram of carboxylate platform.

Conversion of biomass to liquid fuels can occur through three platform: thermochemical, sugar, and carboxylate. In every platform, the methods of biomass conversion and their resultant chemicals are different. Of the three methods of biomass conversion, the sugar and carboxylate platforms are biological. In the *thermochemical platform*, partial combustion of biomass results in significantly lower yields compared to the biological platforms [7]. In the *sugar platform*, biomass is converted to simple sugars and further fermented into ethanol; however, it requires sterile conditions and addition of expensive enzymes [7]. In contrast, in the *carboxylate platform*, polysaccharides are hydrolyzed to sugars that are further fermented to carboxylic acids. Amongst the three platforms, the carboxylate platform is reported to have the highest product yield and lowest cost.

The MixAlco® process is one configuration of the carboxylate platform. It converts biomass to carboxylate salts that can be thermally converted to ketones, hydrogenated to mixed alcohols, and finally oligomerized to hydrocarbons. The feedstock can range from agricultural residues and food scraps to sewage sludge and municipal solid waste. The MixAlco® process employs a mixed culture of microorganisms to produce carboxylic acid from biomass. The advantage of using a mixed culture of microorganisms is that it provides metabolic flexibility and can employ a variety of substrates [8, 9]. In a fermentor, anaerobic digestion occurs in four stages: hydrolysis, acidogenesis, acetogenesis, and methanogenesis [10]. The biochemical changes that occur during each process step are presented in Figure 1-2.

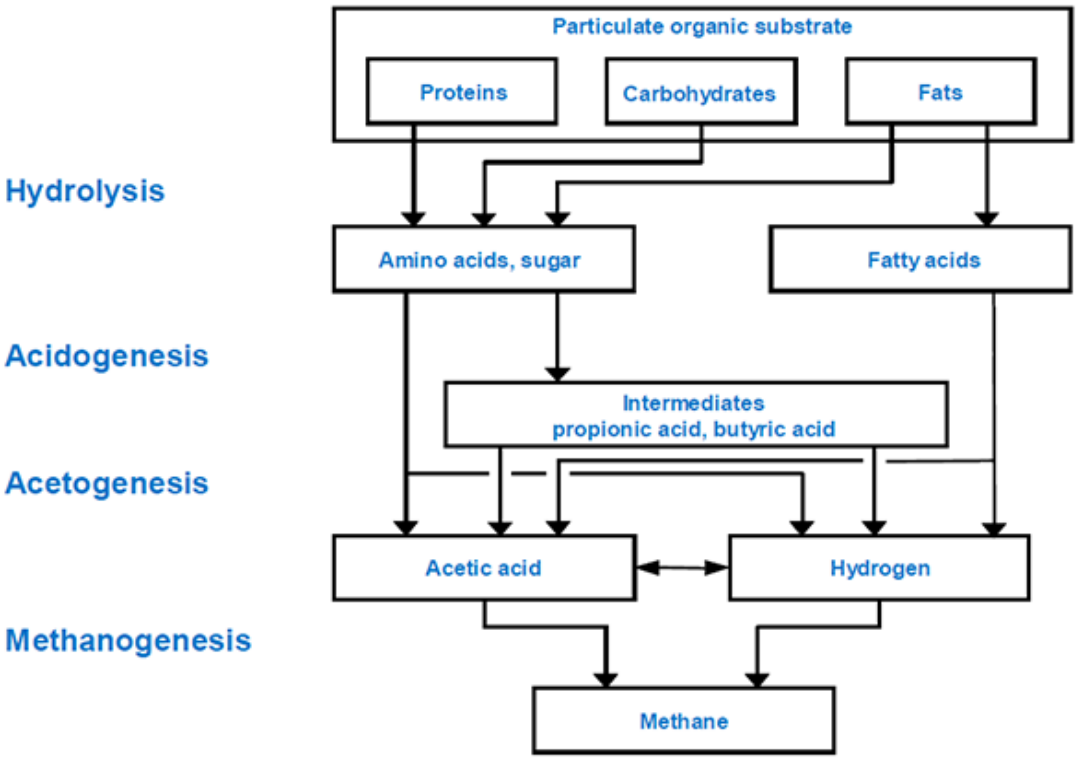


Figure 1-2 Anaerobic digestion process.

Source: Waste-to-Energy Research and Technology Council

Each stage of the anaerobic digestion process is performed by a specific group of microorganisms and is controlled by conditions such as temperature, pH, and substrate source [11]. For example, inhibition of hydrolysis limits the acidogenesis and acetogenesis stages. In the carboxylate platform, methanogenesis is inhibited and biomass that would have otherwise been converted to methane accumulates as carboxylic acids such as acetic, propionic, butyric, valeric, caproic, and heptanoic acids. Inhibition of methane increases acid concentration and decreases pH if insufficiently buffered. Studies have shown that low pH inhibits efficient functioning of

microorganisms in the fermentors. The Continuum Particle Distribution Modeling (CPDM) method developed by Loescher [12] accounts for the inhibitory effect of high acid concentration and its effect on the microbial community in a bioreactor. As the fermentation reaction proceeds and biomass gets digested, its reactivity is reduced and carboxylic acid production rate decreases. Lowering the pH from 7.0 to 6.0 almost completely inhibits cellulose hydrolysis [1]. Thus, in fermentation processes, both the pH and acid concentration are important parameters that affect the conversion and yield.

To improve process efficiency, it is imperative to control the pH and acid concentration in fermentors. Although fermentor pH can be controlled by adding a buffer such as calcium carbonate ($CaCO_3$) or magnesium carbonate ($MgCO_3$), extracting acids from fermentation broth reduces inhibition caused by both high product concentration and low pH. In fermentation broths, most acids are present as deprotonated carboxylate anions; thus, it is preferred to separate deprotonated carboxylate anions rather than the protonated acids themselves. The most common methods for separating carboxylic acids are precipitation, electrodialysis, and liquid-liquid extraction [13]. Of these, precipitation requires large amounts of water and chemicals such as sulfuric acid, calcium hydroxide, etc. Electrodialysis is most effective for low-molecular-weight ionic compounds and is not economically viable when the carboxylate salt concentration is high. Extraction is relatively complex, requires significant amounts of organic solvents, and usually employs membranes for phase separation, which increases costs and maintenance requirements.

Although removing carboxylic acids using ion-exchange resins has been undertaken for a long time, very few studies have focused on improving fermentation rates by removing inhibition caused by high product concentration. Initially, development of ion-exchange processes was conducted in the nuclear industry for isotope separation [14]. Now resins are used to soften water, remove organic acids or heavy metals in wastewater treatment plants, extract and purify food and dairy products, detoxify byproducts in the paper and pulp industry, and to manufacture pharmaceuticals. Low concentrations of carboxylic acids in waste streams of acid manufacturing plants, metal plating industry, boiler feed water, etc. increase chemical oxygen demand (COD) and are considered to be organic pollutants; hence, carboxylic acid removal from aqueous streams is an important problem in various industries. Because large volumes of wastewater with low acid concentration must be treated, ion-exchange resins are usually employed for acid removal [15]. Many studies have been devoted to understanding equilibrium, kinetics, and adsorption characteristics of ion-exchange resins [13, 15-20]. Others have studied adsorption characteristics of specific carboxylic acids for purposes such as acetic acid removal from fuel ethanol [21], recovery of lactic acid from fermentation [22, 23], and applications in food processing industry [24]. Table 1-1 gives a few examples of projects previously undertaken for product recovery in fermentation processes using ion-exchange resins.

Table 1-1 Product recovery in fermentation processes using ion-exchange resins

Purpose	**Culture**	**Ion-exchange resin**	**Reference**
Lactic acid extraction from fermentation of glucose	Pure culture - *Lactobacillus plantarum*	D380, D296, D261, 201×4, 201×2	[25]
Recovery of lactic acid from simultaneous saccharification and fermentation media	Mixed culture - *Lactobacillus delbrueckii* and *Trichoderma reseei* (fungal enzyme)	IRA 900, IRA 400, IRA-96, IRA-67	[26]
Production of hexanoic acid and influence of *in-situ* product removal using ion-exchange resin	Pure culture - *Megasphaera elsdenii*	Amberlite IRA 400	[27]
Removal of acetic acid from fuel ethanol	–	D301R, D330, 201×7, D201	[21]
Removal of acetic acid form spent sulfite liquor (SSL) for xylose fermentation	Pure culture - *Pichia stipitis*	Diaion PA408, Diaion WA30	[28]
Enhance vanillin production from ferulic acid by selective adsorption of vanillin	Pure culture - *Streptomyces* sp. strainV-I	Cad40, CD180, DM11, DM130, HZ803	[29]
In situ separation of lactic acid from fermentation broth using ion-exchange resins	Pure culture - *Lactobacillus casei*	Amberlite resin (IRA-400, Cl^-)	[22]
Improvement of epothilone B production	Pure culture - *Sorangium cellulosum*	XAD-16	[30]
Method of extraction and yield-up of tricycle compounds in fermentation medium	Mixed culture - *Streptomyces venezuelae* and *2'-Streptomyces* sp. GT1005	XAD-2, XAD-4, XAD-7, XAD-7HP, XAD-8, XAD-16, XAD-16HP, XAD-1180, XAD-2000, XAD-2010	[31]

The purpose of this study is to understand the effect of carboxylic acid extraction on fermentation performance using ion-exchange resins. The following is a list of objectives to accomplish this goal:

- Study ion-exchange resin characteristics and understand their behavior in adsorbing carboxylic acids with varying parameters such as pH and acid concentration.
- Perform batch fermentations with different substrates and use ion-exchange resins to extract acids produced on a periodic basis.
- Perform quantitative analysis of fermentation parameters such as conversion, yield, selectivity, and total acid production.
- Summarize key results and extend understanding of batch fermentation extractions to application in countercurrent fermentations.

CHAPTER II

MATERIALS AND METHODS

2.1 Materials

2.1.1 Substrates

Batch fermentations were performed using three different substrates: α-cellulose powder, shredded office paper, and lime-pretreated corn stover. These substrates served as energy source for the microorganisms in the fermentor.

α-Cellulose powder was purchased from Sigma Aldrich (Product number: C8002). α-Cellulose powder was chosen as a substrate for study because cellulose is the most abundant organic polymer and is present in high quantities in lignocellulosic biomass.

Unused office paper was shredded using Fellowes Powershred® W-6C. Tests were conducted to determine the carbon and nitrogen content in paper by Texas A&M University Soil, Water, and Forage Testing Laboratory (College Station, TX). The content of carbon and nitrogen in office paper was found to be approximately 36.03% and 0.07% by weight, respectively.

Corn stover used as substrate for the batch fermentation underwent submerged lime pretreatment (SLP) to remove lignin and improve digestibility. The pretreatment was performed by mixing 2.7 kg corn stover, 180 mL water, and 3 g lime ($Ca(OH)_2$) in a 60-L jacketed vessel and kept at 50˚C for 4 weeks [3]. Once pretreatment was completed, excess lime was removed by washing the biomass with dilute hydrochloric

acid (HCl) until the pH dropped to 4.5 followed by a wash with water to remove residual acid. Carbon and nitrogen content in lime-pretreated corn stover was found to be 39.85% and 0.13% by weight, respectively.

2.1.2 Nutrients

Chicken manure, a source of nutrients and minerals for the fermentations, was obtained from Feather Crest Farms, Inc. (Bryan TX). Wet chicken manure degrades over a period of time and therefore was dried and homogenized to maintain consistency throughout the experiment [3]. Wet chicken manure was air dried at 105˚C for 48 h to reduce its moisture content ≤ 10%. It was then transferred to Ziploc air-tight plastic bags and stored until use. Carbon and nitrogen content in chicken manure was 28.17% and 2.22% by weight, respectively, and the acid concentration was 0.05 ± 0.01 g acid/g dry chicken manure. In the fermentors, urea (Fisher Scientific, Product no. U15-500) was added as a source of nitrogen to adjust the carbon-nitrogen ratio. The target carbon-nitrogen ratio for the fermentations was 25 g carbon/g nitrogen.

2.1.3 Fermentor configuration

Batch fermentations were performed in 1-L polypropylene plastic bottles with a rubber stopper inserted with a septum-covered glass tube [32]. The rubber stopper had two stainless steel tubes inserted, which aided mixing of fermentor contents as it rotated in the incubator. Figure 2-1 shows a cross-sectional view of the fermentor. The temperature of the incubator was constantly maintained at 40˚C

.

2.1.4 Fermentation media

To prepare the fermentation media, distilled water was boiled to liberate dissolved oxygen and after cooling to room temperature in a covered vessel, 0.275 g/L cysteine hydrochloride and 0.275 g/L sodium sulfide were added to further reduce the oxygen content.

2.1.5 Iodoform

Methane production in fermentors was inhibited by using iodoform (CHI_3). Every 48 h, 120 µL of iodoform solution (20 g CHI_3/L, 190-proof ethanol) was added to each fermentor. Iodoform is sensitive to light, air, and temperature; hence, the solution was kept in an amber-colored glass bottle wrapped in foil and stored at −20˚C until use. The bottle cap was replaced immediately after use to prevent degradation. Iodoform was added every 48 h until the 12^{th} day of the fermentation. After that, it was added only if there was a methane peak observed in the gas chromatograph of the headspace gas sample.

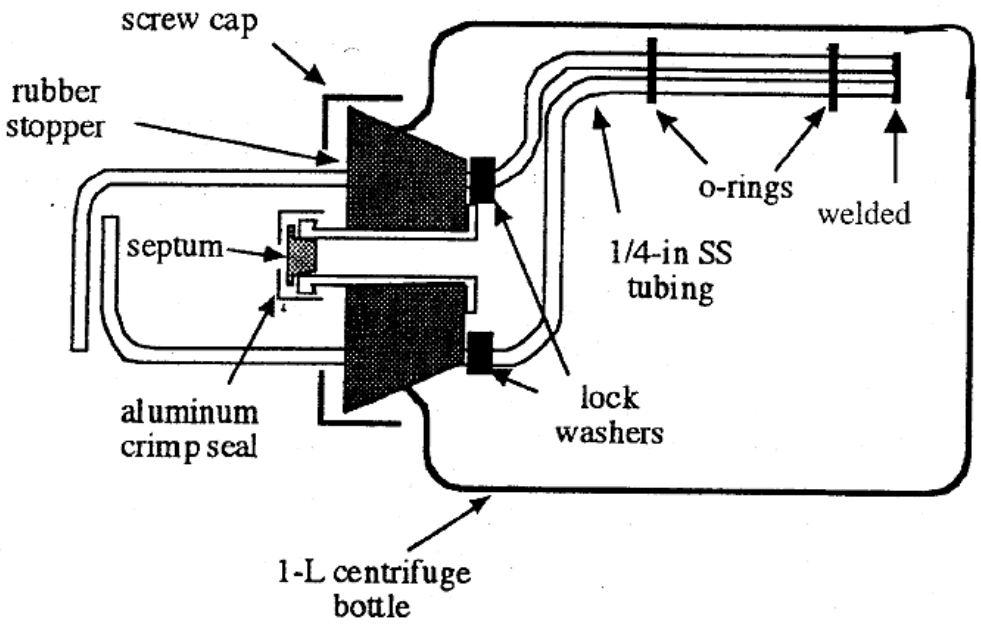


Figure 2-1 Polypropylene fermentor.

2.1.6 Inoculum

A mixed culture of marine microorganisms was collected from beach sediments at Galveston Island, TX. Sediments were collected from the bottom of 0.5-m-deep shoreline pits. To minimize exposure to oxygen, the sediment collected was immediately placed in airtight plastic bottles filled with deoxygenated water, 0.275 g/L cysteine hydrochloride, and 0.275 g/L sodium sulfide. All samples collected were capped and frozen at −20˚C until use.

2.1.7 Buffer

In the control fermentors, magnesium carbonate ($MgCO_3$) was used to buffer the fermentation broth and continuously maintain a pH in the near-neutral range.

Magnesium carbonate is preferred over calcium carbonate because it provides a tighter pH control in the near-neutral range.

2.1.8 Ion-exchange resins

A known quantity of Amberlite® IRA-67 ion-exchange resin (Alfa Aesar, Product No. 42253) was washed with water to remove impurities and excess amines. Excess water was removed by vacuum filtering the resins. Moisture content of resins was determined by two methods. A sample of resin (5 g) was placed in a convection oven at 105˚C. The difference in mass of a sample before and after heating in the oven was noted and the percentage by mass of moisture in resin was calculated. The moisture content of resins was also tested using a moisture analyzer (Denver Instruments, IR 120). The device monitored the mass percentage of moisture in the resin as the moisture evaporated out of the resin. Washed resins were used in column mode to extract acid from the fermentation broth, thereby maintaining the pH in the desirable range of 6.8−7.2.

2.2. Methods

2.2.1 Biogas analysis

Biogas is continually formed in the fermentors; therefore, it must be removed periodically to reduce pressure buildup and prevent fermentor rupture. For the first 10 days of fermentation, each fermentor was monitored daily and thereafter each fermentor was vented every 48 h. Biogas was removed by puncturing a needle through the fermentor septum. A needle was fitted with a valve to control air flow through it and was connected to a well-sealed inverted cylinder by a polypropylene tube. The inverted

graduated glass cylinder was filled with an aqueous solution of 300 g CaCl/L to prevent microbial growth and carbon dioxide adsorption [3]. Because iodoform is added to the fermentors to prevent methane formation, a 5-mL gas sample was collected periodically to check for the presence of methane. The gas sample was passed into an Agilent 6890 Series chromatograph with a thermal conductivity detector (TCD). A 4.6-m stainless steel packed column with 2.1-mm ID (60/80 Carboxen 100, Supelco I-2390) was used. The inlet temperature was 230°C, whereas the detector and oven temperature were 200°C. Total run time was 20 min and helium was the gas carrier. The GC was calibrated as shown in Table 2-1.

2.2.2 Carboxylic acid concentration determination

Fermentation liquid was collected periodically for acid analysis. Reactors were centrifuged (4,000 rpm, 25 min), mixed with equal parts of internal standard (1.162 g/L 4-methyl-n-valeric acid) and 3-M phosphoric acid (H_3PO_4), and finally ultra-centrifuged (15,000 rpm, 8 min). The H_3PO_4 added during GC sample preparation ensured that deprotonated carboxylate anions are converted into protonated carboxylic acids prior to analysis. Total carboxylic acid concentrations (protonated and deprotonated) were measured using an Agilent 6890 Series gas chromatograph (GC) system equipped with a flame ionization detector (FID) and an Agilent 7683 automatic liquid sampler. A 30-m fused-silica capillary column (J&W Scientific Model # 123-3232) was used. The column head pressure was maintained at 2 atm abs. The gas sample calibration table is given in Table 2-1.

Table 2- 1 Gas sample calibration table

Retention Time	**Signal**	**Amount**	**Area**	**Amt/Area**	**Ref**	**Group Name**
[min]		[%]				
1.154	2.00	1	1.0200	1370.57	0.0007	Hydrogen
3	5.01	3428	0.0015			
4	15.00	10357	0.0014			
2	50.00	34483	0.0014			
13.817	1.00	1	1.0000	794.98	0.0013	CO2
3	5.00	4362	0.0011			
4	15.00	13258	0.0011			
2	50.00	44845	0.0011			
17.188	1.00	1	0.5000	374.02	0.0013	Oxygen
7	23.97	18302	0.0013			
5	85.02	65578	0.0013			
6	95.01	73485	0.0013			
17.578	1.00	6	4.9900	3979.08	0.0013	Nitrogen
1	6.00	5102	0.0012			
5	14.98	12221	0.0012			
4	55.00	44512	0.0012			
7	76.03	62161	0.0012			
3	84.99	67580	0.0013			
19.086	1.00	3	5.0000	3264.59	0.0015	Methane
4	15.00	9788	0.0015			
1	69.00	43381	0.0016			

After each sample injection, the GC temperature program raised the temperature from 40°C to 200°C at 20°C/min. The temperature was subsequently held at 200 °C for 2 min. The total run time per sample was 15 min. Helium was used as the carrier gas. The external standard was a volatile acid mix (Matreya, LLC, Cat No. 1075), used to calibrate the samples against the IC-6 internal standard. The concentration of carboxylic acids in the external standard is given in Table 2-2.

Table 2- 2 Carboxylic acid calibration in external standard

Acid	**Amount (g/L)**
Acetic acid	4.0132
Propionic acid	3.0136
Iso-butyric acid	0.9924
Butyric acid	1.9789
Iso-pentanoic acid	0.7976
Pentanoic acid	1.5471
Iso-hexanoic acid	1.1618
Hexanoic acid	0.808
Heptanoic acid	0.3952
Octanoic acid	0.1698
Succinic acid	1.0001

2.2.3 Moisture and volatile solid content analysis

Moisture content and ash content of the substrates were determined as described in NREL procedures [3, 33]. Ash content was calculated on a dry basis. For accurate measurement of volatile acids produced, 30 mg $CaOH_2$/(g sample) was added to the sample before placing it in the oven at 105°C. Addition of calcium hydroxide to the sample before drying ensured conversion of all volatile acids to their deprotonated forms. *Moisture content* [4] in biomass is defined as g water/g biomass. Substrates were heated at 105ºC for 24 h in a conventional oven and the difference in weight of sample was noted. The consumption of non-acid volatile solids (NAVS) was determined using the inert-ash approach [34]. *Volatile solids* (VS) are defined as the mass of dry solid material that is combusted at 550°C for 24 h. The residual solids remaining after heating the sample at 550°C for 24 h is known as *ash*. NAVS is defined as the difference between mass of VS and mass of carboxylic acid present [3].

$$\text{NAVS} = [(1-\text{MC}) \times (1-\text{ash}) \times \text{Total biomass (g)}] - (\text{g total carboxylic acid}) \quad (2\text{-}1)$$

2.2.4 Inocula adaptation

Three sets of fermentations were started with α-cellulose, office paper, and lime-pretreated corn stover as biomass and using the same fresh inocula, chicken manure, urea, and deoxygenated water to allow microorganisms to adapt to fermentation conditions and establish a culture. On the 15th day, all fermentors were centrifuged and the liquid and solid fractions were separated. The liquid fraction was stored and used as inoculum for the batch fermentation.

2.3. Fermentation performance parameters

The performance of the fermentors was measured by parameters such as conversion, yield, and selectivity. The data collected through the entire run of the experiment was used to calculate the performance parameters. Figure 2-2 shows the conversion of various components of biomass to final products in solid, liquid, or gas phase. The desired product of this process is carboxylic acids; hence, non-acid volatile solids (NAVS) was used as measure of biomass when calculating fermentation parameters. Chicken manure contains some carboxylic acids and this amount is considered when calculating the total acids produced. In Figure 2-2, a component for water of hydrolysis is included because carbohydrates are broken down into simple sugars by hydrolysis. Furthermore, $MgCO_3$, which is added to the fermentations as a buffer, reacts with the H^+ ions (of the carboxylic acid groups) in the fermentation broth to produce water molecules, known as water of neutralization. Hence, the products include a component of water of neutralization. Acetic acid equivalents (Aceq.) are defined later in Eq. 4-6 and 4-7.

$$\text{Volatile solids (VS)} = \text{Dry weight} - \text{Ash weight} \tag{2-2}$$

where, dry weight is measured after heating the sample at 105˚C and ash weight is measured after heating the sample at 550 ˚C.

$$\text{Conversion} = \frac{\text{NAVS}_{\text{digested}}\ (\text{g})}{\text{NAVS}_{\text{fed}}\ (\text{g})} \tag{2-3}$$

$$\text{Yield} = \frac{\text{Total carboxylic acids produced (g)}}{\text{NAVS}_{\text{fed}}\ (\text{g})} \tag{2-4}$$

$$\text{Yield}_{(\text{Aceq})} = \frac{\text{Total carboxylic acids produced (in Aceq.)(g)}}{\text{NAVS}_{\text{fed}}\ (\text{g})} \quad (2\text{-}5)$$

$$\text{Selectivity} = \frac{\text{Total carboxylic acid produced (g)}}{\text{NAVS}_{\text{digested}}\ (\text{g})} \quad (2\text{-}6)$$

$$\text{Selectivity}_{\text{Aceq}} = \frac{\text{Total carboxylic acid produced (in Aceq)(g)}}{\text{NAVS}_{\text{digested}}\ (\text{g})} \quad (2\text{-}7)$$

$$\text{NAVS}_{\text{digested}} = \text{NAVS}_{\text{fed}} - \text{NAVS}_{\text{remaining}} \quad (2\text{-}8)$$

$\text{NAVS}_{\text{remaining}}$ = VS in separated liquid + VS in cake – Total acid (liquid + cake) (2-9)

where VS in cake includes VS in liquid in cake and VS of dry cake solids

$$\text{NAVS}_{\text{fed}} = \text{VS}_{\text{chicken manure}} + \text{VS}_{\text{biomass}} + \text{VS}_{\text{urea}} - (\text{Initial acid present}) \quad (2\text{-}10)$$

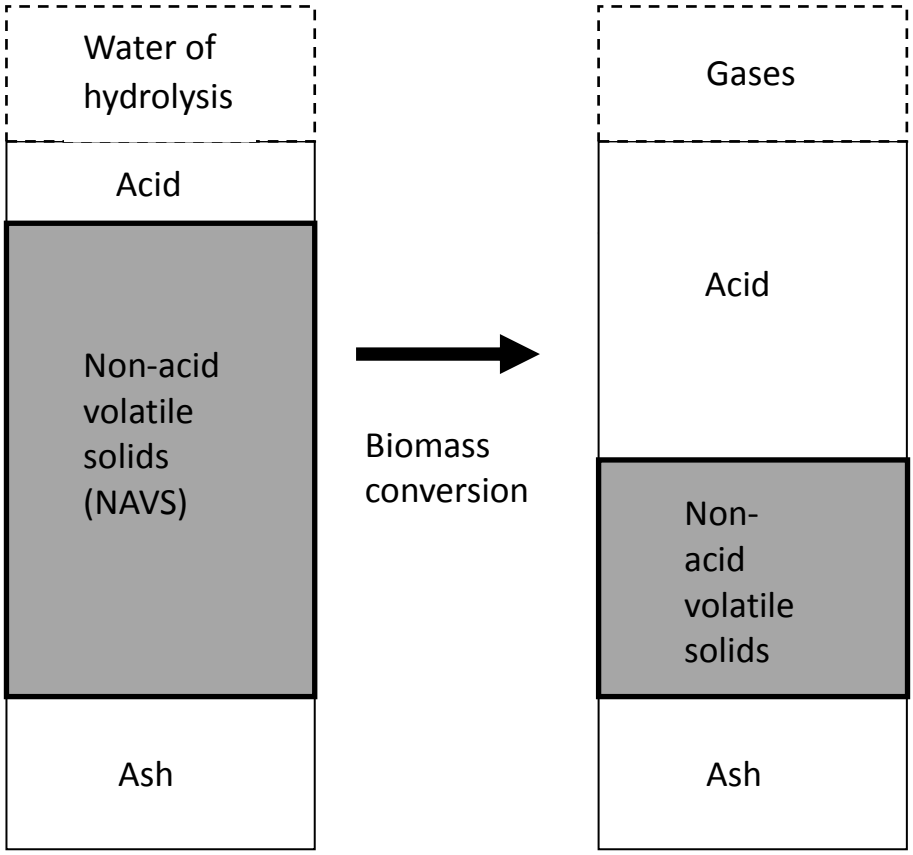


Figure 2-2 Biomass conversion.

CHAPTER III

ION-EXCHANGE RESINS

3.1 Overview

Ion-exchangers are defined as substances that carry replaceable ions. Ion-exchangers can be natural (e.g., zeolites, clay, montmorillionite) or artificial (e.g., functional polymers, ion-exchange resins) [14]. Substances that carry exchangeable positively charged ions are *cation exchangers*. Those that carry exchangeable negatively charged ions are *anion exchangers*. Those that simultaneously carry positive and negative charged ions are *amphoteric exchangers*. The three-dimensional matrix of an ion-exchange resin is comprised of linear polymeric chains containing active functional sites such as $–SO_3H$, –COOH, -N, $-NH_2$. Depending on the chemical behavior of the functional group, resins can be categorized as strong acid, strong base, weak acid, or weak base ion exchange resins. Thus, in ion-exchange resins [1], functional groups can be acidic, basic, or chelating in nature. The polymeric chains in ion-exchange resins are held together by short hydrocarbon bridges known as cross links. Crosslinking in resins imparts stability to the resin structure and the density of cross links in a resin is known as the *degree of crosslinking*. If the degree of crosslinking is high, the resin is hard and stable. However, a high degree of crosslinking reduces the elasticity of the resin causing low diffusion rates because the resin does not expand much to allow molecules to enter into the adsorption sites that are inside the pores [14]. Figure 3-1 shows a schematic of cross-sectional views of anionic and cationic resins.

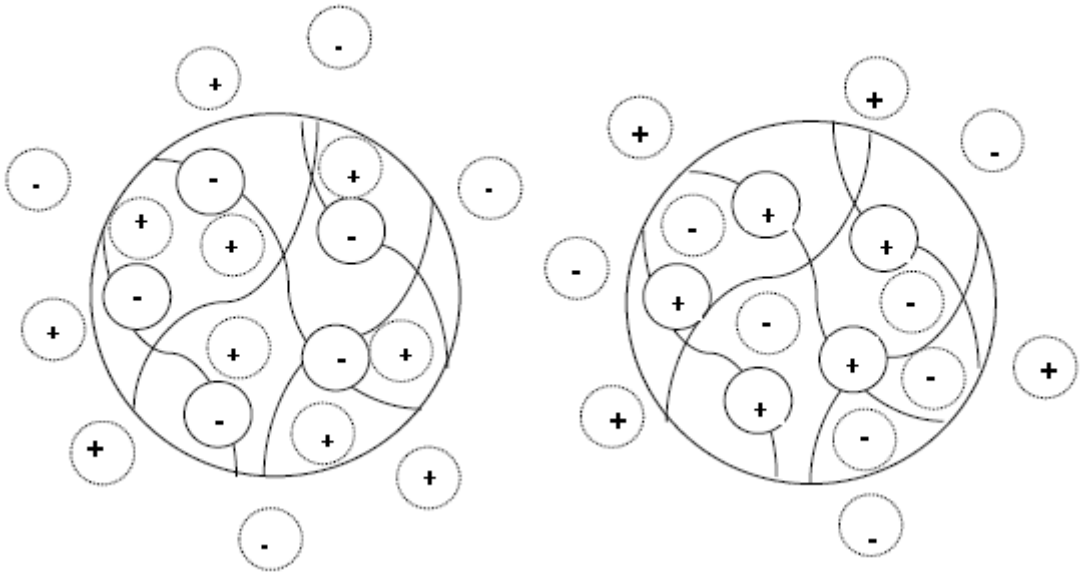


Figure 3- 1 Schematic of cross-sectional views of a) anionic resin b) cationic resin.

Initially, ion-exchange processes were developed for the nuclear industry to separate isotopes [14]. Now, resins are used to soften water, remove organic acids or heavy metals in wastewater treatment plants, extract and purify food and dairy products, detoxify byproducts in the paper and pulp industry, and to manufacture pharmaceuticals. Low concentrations of carboxylic acids in waste streams of acid manufacturing plants, metal plating industry, boiler feed water, etc. increase chemical oxygen demand (COD) and are considered to be organic pollutants; hence, carboxylic acid removal from aqueous streams is an important problem in various industries. Because large volumes of wastewater with low acid concentration must be treated, ion-exchange resins are usually employed for acid removal [15].

In fermentation broth, acids (e.g., CH_3COOH) are usually present in their deprotonated carboxylate anion form (e.g., CH_3COO^-). When fermentation broth passes through a weak-base anion-exchange resin column, the sorption of acid occurs in two steps [17]. The H^+ ions protonate free amine groups of the resin as described:

$$H^+ + :NR_3 \rightarrow H^+NR_3 \quad (3\text{-}1)$$

Protonation of the amine group forms a positive charge on the surface of the pore walls of the resin. Electrostatic interaction leads to anion association between the positively charged H^+NR_3 and negatively charged carboxylate salts [17].

$$H^+NR_3 + Ac^- \rightarrow H^+NR_3\,(Ac^-) \quad (3\text{-}2)$$

Regeneration of acids can be accomplished by passing a strong base such as NaOH or $Ca[28]_2$ through the saturated anion-exchange resins allowing the following reaction to occur:

$$H^+NR_3\,(Ac^-) + NaOH \rightarrow :NR_3 + H_2O + Na^+ + Ac^- \quad (3\text{-}3)$$

When selecting resins for application in the MixAlco® process, the most important properties considered were the sorption capacity and the ease of regeneration. The efficiency of resins to adsorb acids is usually expressed as *total exchange capacity*. *Total exchange capacity* is defined as the total sites available for exchange on the resin per amount of resin and is expressed using units such as meq. acid adsorbed/mL or meq. acid adsorbed/g resin. Reversibility of ion exchange depends on the type of acid adsorbed and basicity of the resin, which can be classified as being high, medium, or weak [15]. Resins with high basicity exhibit high sorption capacity even at pH values much higher than the pK_a of acid in solution. However, high sorption capacity does not

necessarily indicate low reversibility. For example, weak acids such as acetic acid showed a high degree of reversibility when adsorbed on resins having high basicity. Bhandari *et al*. also correlated basicity with resin type and inferred that polyacrylic polyfunctional resins exhibited high basicity. In contrast, polystyrene monofunctional and polyfunctional resins exhibited medium basicity. Thus, when selecting resins for application in the MixAlco® process, the resin material was also considered as an important factor.

Amberlite IRA-67 and Amberlite IRA-96 were chosen for a comparative study. Amberlite IRA-67 is a weak-base anion-exchange resin with a gel-type structure and acrylic matrix with divinylbenzene as copolymer whereas Amberlite IRA-96 is a weak-base anion-exchange resin of macroreticular structure and styrene divinylbenzene copolymer matrix. The functional group of both the resins is the tertiary amine group ($:NR_3$). The physical and chemical properties of ion-exchange resins IRA-67 and IRA-96 are outlined in Table 3-1.

Table 3-1 Properties of Amberlite IRA-67 and Amberlite IRA-96

Characteristics	**IRA-67**	**IRA-96**
Form	Translucent white spherical beads	Tan opaque spherical beads
Matrix	Acrylic−DVB	Styrene–DVB
Structure	Gel	Macroporous
Functional group	Tertiary amine	Tertiary amine
Capacity (eq./L)	1.6	1.25
Particle size [24]	0.5−0.75	0.55−0.75
Moisture content (%)	56−64	57−63

3.2 Experimental method

3.2.1. Moisture content determination

Physical examination of the ion-exchange resins indicated that the resins were not free flowing, but lumped together mostly because of moisture on resin surface. The moisture bound to the resin could be categorized as either free moisture (present on the surface of the resin) or bound moisture (bound to the polymeric chains in the resin). Product data sheet supplied by the manufacturers for Amberlite IRA-67 and Amberlite IRA-96 stated that the moisture content was 54–67%. This study was conducted to determine the total moisture content of IRA-67 and IRA-96. Resin samples were washed 2−3 times with DI water, vacuum filtered to remove excess moisture, transferred to a glass bottle, and capped until use. Two different methods were used to determine percent moisture in resins on mass basis. In the first method, about 5 g of wet resin was placed in a crucible, weighed, and inserted into an oven at 105˚C. After 24 h, the crucible was removed from the oven, cooled in a desiccator, and its final weight was noted. In the second method, moisture content of resin samples was measured using a moisture content analysis device (Denver Instruments, IR 120). In this apparatus, a weighed amount of resin was heated to 105˚C and the mass percentage of moisture in resin was measured as it continually evaporated from the resin. Moisture content of resins was used to calculate the dilution effect in batch experiments for acid adsorption capacity.

3.2.2. Acid adsorption capacity

This study was undertaken to determine the acid adsorption capacities of Amberlite IRA−67 and Amberlite IRA−96 expressed as meq acetic acid adsorbed/g dry

resin and meq acetic acid adsorbed/g wet resin. Six centrifuge tubes were filled with 20 mL of 1-M acetic acid solution and the initial pH of each sample was noted. Out of the six centrifuge tubes, three tubes were filled with Amberlite IRA-67 (4 g, wet resin mass basis) and the remaining three were filled with Amberlite IRA-96 (4 g, wet resin mass basis). The test tubes were then placed in a shaking apparatus maintained at room temperature.

Initial concentration of the acetic acid solution prepared was analyzed by a gas chromatograph (GC) system equipped with a flame ionization detector (FID) and an Agilent 7683 automatic liquid sampler. The pH of the samples were measured on a time interval basis of 12 h for a period of four days. pH changes were negligible after 36 h and the system was considered to be in equilibrium. The final pH of each sample was measured at the end of four days and a 0.5-mL sample was drawn from each tube for GC analysis. The difference in initial and final acid concentration was assumed to have been completely adsorbed by the ion-exchange resins.

Moisture content [4] analysis of resins indicated that moisture in resin could significantly dilute the acid solution and was therefore considered while calculating acid adsorption capacity of resins.

$$V_{final} = V_{initial} + \frac{MC \times W}{\rho} \tag{3-4}$$

where V is volume of solution (mL), MC is the moisture content of the resin (expressed as fraction by mass), and W is the wet mass of resin (g). The density of water (ρ) was assumed to be 1 g/mL at room temperature

.

Calculations

Substituting moisture content values from Table 3-3 in Eq. 3-1, we get V_{final} for each sample, as shown in Table 3-5. After measuring the final pH, a 0.5-mL sample was drawn from each centrifuge tube for GC analysis. The difference in initial and final acid concentration was assumed to have been completely adsorbed by the ion-exchange resins.

$$\text{Acid adsorbed by resin (g)} = \left(C_{initial} \times \frac{V_{initial}}{1000}\right) - \left(C_{final} \times \frac{V_{final}}{1000}\right) \quad (3\text{-}5)$$

where C is the acid concentration (g/L). Acid adsorption capacity of resins can then be expressed in terms of mass dry resin or mass wet resin and is calculated as follows:

$$\text{Acid adsorption capacity}_{wet} = \frac{\text{Acid adsorbed (g)}}{\text{Resin mass}_{wet}\text{ (g)}} \quad (3\text{-}6)$$

$$\text{Acid adsorption capacity}_{dry} = \frac{\text{Acid adsorbed (g)}}{\text{Resin mass}_{dry}\text{ (g)}} \quad (3\text{-}7)$$

3.2.3. Resin adsorption characteristics in mixed acid systems

The purpose of this study was to analyze carboxylic acid adsorption characteristics of Amberlite IRA-67 and Amberlite IRA-96, and compare their acid adsorption efficiency. To simulate fermentation broth, 2 L of 22.20 g/L carboxylic acid solution was prepared. A study of adsorption of the following carboxylic acids believed present in the fermentation broth was conducted: acetic, propionic, isobutyric, butyric, isovaleric, valeric, and hexanoic acids. High-molecular-weight carboxylic acids such as heptanoic and octanoic acids were disregarded, because their concentrations in

fermentation broth are usually low. The constituents of the carboxylic acid solution used in these experiments are summarized in Table 3-2.

A measured quantity of acid solution was diluted with deionized water in a 1−L volumetric flask. A NaOH solution (50% w/w) was prepared to adjust the pH of the acid solution. Mixing sodium hydroxide with water is an exothermic reaction, hence NaOH was added incrementally to deionized water while stirring constantly. Of the carboxylic acid solution, 900 mL was transferred into a large glass beaker. The beaker was placed on a magnetic stir plate (Thermo Scientific, Model no. HP133730-33Q). A magnetic stir bar placed in the beaker ensured continuous mixing of liquid while a pH electrode inserted in the liquid allowed for constant pH measurement. NaOH solution was added drop-wise to the acid solution in the beaker using a 5-mL transfer pipette. Once the desired pH was reached, 80 mL of the solution was drawn and stored in a 250-mL polypropylene bottle. Two identical sets of carboxylic acid solutions of 20 mL each were made by varying the solution pH for resins IRA-67 and IRA-96.

The theoretical amount of resin required to completely adsorb the acid in each solution was calculated using the solution concentration and the acid adsorption capacity as outlined in Eq. 3-3. The actual resin amount added to each centrifuge tube was 0.5 g more than the theoretical amount calculated. The pH of each sample was measured and a 0.5-mL liquid sample was drawn from each tube for gas chromatograph (GC) analysis. Centrifuge tubes were then sealed and placed in an orbital shaking incubator maintained at room temperature for 3 days.

Table 3-2 Carboxylic acid constituents

Carboxylic acid	**Concentration (g/L)**
Acetic acid	7.365
Propionic acid	6.430
Isobutyric acid	0.511
Butyric acid	2.595
Isovaleric acid	0.705
Valeric acid	2.690
Hexanoic acid	1.901
Total	**22.197**

3.2.4. Effect of equilibrium acid concentration on adsorption

The purpose of this study was to understand the effect of equilibrium concentration and pH on acid adsorption. Acid solutions of 10, 20, and 30 g/L for acetic, propionic, and hexanoic acids were prepared using 99.8% pure acid. Initial acid concentration of each solution prepared was measured using an Agilent 6890 Series gas chromatograph (GC) system equipped with a flame ionization detector (FID) and an Agilent 7683 automatic liquid sampler. Sodium hydroxide (NaOH) solution (50% w/w) was prepared to adjust the pH of the acid solution. Mixing NaOH with water is an exothermic reaction; hence, it was added incrementally to deionized water while stirring constantly. Ion-exchange resin IRA-67 was washed with deionized water 2−3 times to remove excess impurities and free amines and stored in a glass bottle until used. Moisture content of resins was determined as described in Section 3.1.1.

Acid solution (300 mL) was transferred in a 500-mL glass beaker, which was placed on a magnetic stir plate. The magnetic stir bar placed in the beaker ensured continuous mixing of liquid while the pH electrode inserted in the liquid allowed for constant pH measurement. NaOH solution was added drop-wise to the acid solution in the beaker using a transfer pipette. Once the desired pH was reached, 20 mL of solution was drawn from the beaker and stored in a 50-mL centrifuge tube. This procedure was repeated for different initial acid concentrations of acetic, propionic, and hexanoic acids.

The theoretical amount of resin required for complete adsorption of acid in each solution was calculated using the solution concentration and the acid adsorption capacity as outlined in Eq. 3-3. The actual resin amount added to each centrifuge tube was 0.5 g more than the theoretical amount calculated. The centrifuge tubes were then sealed and placed in an orbital shaking incubator maintained at room temperature for 3 days. The continuous shaking allowed for optimum contact between the resins and acid solution leading to the system quickly attaining equilibrium at the end of 3 days. The final pH of solution at the end of the third day was recorded and a 0.5-mL liquid sample was drawn from each tube for gas chromatograph (GC) analysis. The GC results indicated the amount of acetic, propionic, butyric, valeric, and hexanoic acids in each sample once it had reached equilibrium. The difference between the initial acid concentration and final acid concentration was assumed to be completely adsorbed by the resins. The dilution effect due to moisture from the resin was incorporated when calculating the amount of acid adsorbed onto the resin. The percentage of acid adsorbed by the resin with respect to maximum resin adsorption capacity was calculated using Eq. 3-5.

$$\% \text{ Adsorption capacity} = \frac{\text{Acid bound to resin (g)}}{\text{Resin capacity(g acid/g wet resin)} \times \text{Wet resin (g)}} \quad (3\text{-}8)$$

3.2.5. Ion-exchange resins in fixed-bed column operation

The advantage of using ion-exchange resins lies in the ability to regenerate resins when their adsorption capacity decreases. This study was undertaken to identify operation parameters for ion-exchange resin Amberlite IRA-67 in column mode. Resins were poured into a 100-mL glass column (Pyrex, No. 32152). Acid solution was continuously passed through an ion-exchange resin column and flow was ceased only when the column had reached its maximum adsorption capacity and was not adsorbing any more acid. Acids adsorbed onto the resin were recovered easily by passing a strong base such as calcium hydroxide ($Ca(OH)_2$) or sodium hydroxide (NaOH) through the column. Three solutions were prepared containing 20 g/L of acetic, propionic, or hexanoic acids. A peristaltic pump was used to pass acid solution into the ion-exchange resin column and the outlet stream of solution was collected at 10-mL intervals for the first 200 ml of acid solution passed. Acid concentration of each sample was analyzed using a gas chromatograph (GC). When the column had reached its maximum adsorption capacity and required regeneration, 1-M NaOH solution was passed through the resin column and the solution eluted from the column was collected in 20-mL intervals. The volume of NaOH solution to be passed through the resin column was calculated as 240 mL, 280 mL, and 260 mL for acetic acid, propionic acid and hexanoic acid solutions respectively. Samples of the solution eluted were prepared for acid concentration analysis by GC.

3.3 Results and discussions

3.3.1 Moisture capacity

The total moisture contents for ion-exchange resins Amberlite IRA-67 and Amberlite IRA-96 are presented in Table 3-3 and Table 3-4. The product data sheets indicate the moisture content to be 56−64% for both IRA-67 and IRA-96 and the moisture content calculated from the experimental results falls within this range. The product data sheets for both resins specified a maximum operational temperature of 60˚C and heating the resins to 105ºC changed their color from white to yellowish orange. According to the conventional oven method, the moisture content for Amberlite IRA-67 was calculated to be 57.79 ± 0.04% and for Amberlite IRA-96 was calculated to be 58.52 ± 0.47%. Moisture content analysis device measurements were 57.58% and 58.63% for Amberlite IRA-67 and Amberlite IRA-96, respectively.

Table 3-3 Moisture content using conventional oven method

Sample	Resin (g)	Dry resin weight (g)	Moisture (g)	Moisture (% by mass)
IRA-67	5.11	2.16	2.95	57.76
IRA-67	5.07	2.14	2.93	57.81
IRA-96	4.92	2.05	2.86	58.20
IRA-96	4.98	2.05	2.93	58.83

Table 3-4 Moisture content analysis device

Sample	Moisture content (% by mass)
IRA-67	57.58
IRA-96	58.63

3.3.2 Acid adsorption capacity

The final concentration of samples in 50-mL centrifuge tubes were analyzed by a GC equipped with a flame ionization detector (FID) and an Agilent 7683 automatic liquid sampler. Acid adsorption capacity was calculated as explained in Section 3.2.2. The average acid adsorption capacity for IRA-67 in the dry and wet phase was 0.385 ± 0.005 g acetic acid/g dry resin and 0.164 ± 0.002 g acetic acid/g wet resin, respectively. The acid adsorption capacity of the resin can also be expressed as eq/L. To convert the acid adsorption capacity of resins in wet phase to eq/L, the numerator i.e. grams of acid adsorbed was converted to equivalents of acid adsorbed and using the moisture content of the resins, the denominator i.e. mass of water in resins was converted to volume of water in resins (L). For IRA 67, the product data sheet listed the resin capacity as ≥1.6 eq/L in free base form and the calculated value was 4.65 eq/L for wet resin. The average acid adsorption capacity for IRA-96 in the dry and wet phase was 0.33 ± 0.006 g acetic acid/g dry resin and 0.14 ± 0.002 g acetic acid/g wet resin respectively. For IRA 96, the product data sheet listed the resin capacity as ≥1.25 eq/L in free base form and the calculated value was 3.95 eq/L for wet resin. The results are listed in Table 3-5.

Table 3-5 Acetic acid adsorption capacity

Initial concentration: 58.7153 g/L						
Sample	**Dry resin mass (g)**	**Wet resin mass (g)**	**Final Concentration (g/L)**	V_{final}	**Acetic acid capacity$_{dry}$ (g acid/g dry resin)**	**Acetic acid capacity$_{wet}$ (g acid/g wet resin)**
IRA-67 – a	2.00	4.72	20.27	22.71	0.384	0.163
IRA-67 – b	2.12	4.99	17.25	22.87	0.392	0.166
IRA-67 – c	2.04	4.81	19.82	22.77	0.381	0.162
IRA-96 – a	2.17	5.10	22.19	22.93	0.337	0.143
IRA-96 – b	2.08	4.91	24.84	22.82	0.325	0.138
IRA-96 – c	2.06	4.86	24.87	22.80	0.328	0.139

3.3.3 Resin adsorption characteristics in mixed acid systems

The values for percent acid adsorbed for individual acids and total acid for Amberlite IRA-67 and Amberlite IRA-96 are summarized in Table 3-6 and Table 3-7, respectively. Fig 3-2 graphically compares the total mass of carboxylic acid adsorbed per mass wet resin at various equilibrium pH values for the two systems. Amberlite IRA-67 showed a higher loading than Amberlite IRA-96. From Table 3-6 it is evident that Amberlite IRA-67 adsorbed high-molecular-weight carboxylic acid more efficiently than the low-molecular-weight carboxylic acids for all measured equilibrium pH values. Selective extraction of high-molecular-weight acids is advantageous for the MixAlco® process because the heavier acids are more readily converted to fuel. Amberlite IRA-67 was selected to be used for carboxylic acid extraction from fermentation broth because it has a much higher adsorption capacity than Amberlite IRA-96 and also shows selectivity for high-molecular-weight acids.

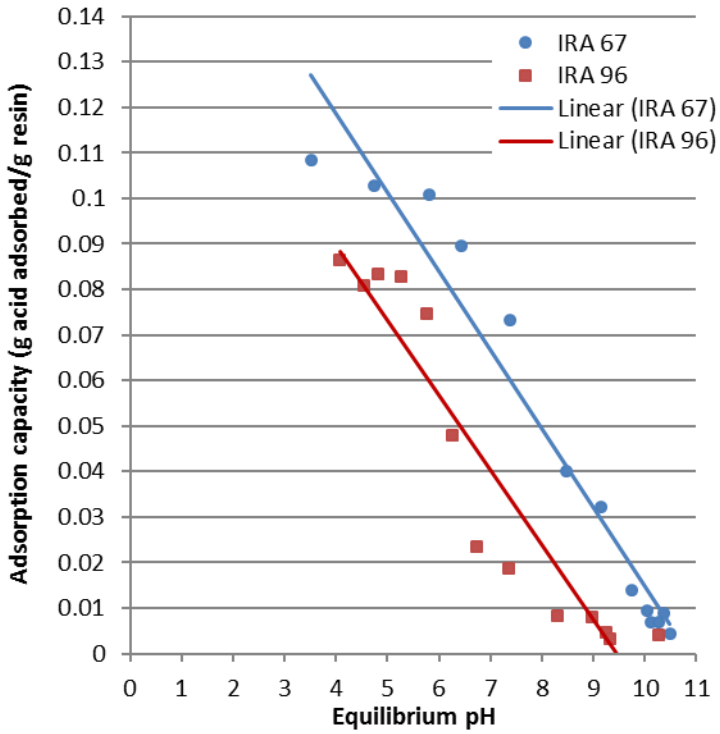


Figure 3- 2 Comparison of adsorption capacity of IRA-67 and IRA-96.

Table 3-6 Percent acid adsorbed − Amberlite IRA-67

Initial pH	Final pH	C2 (%)	C3 (%)	IC4 (%)	C4 (%)	IC5 (%)	C5 (%)	C6 (%)	Total acids (%)
2.76	3.52	97.71	97.55	97.26	96.84	97.84	98.12	98.31	97.66
2.99	4.73	93.02	93.21	92.18	92.86	91.63	92.66	92.24	92.54
3.49	5.81	90.41	91.39	90.47	90.74	88.07	92.61	91.43	90.73
3.95	6.44	79.90	79.76	78.33	80.61	81.46	82.21	82.91	80.74
4.49	7.39	65.62	63.22	58.29	62.72	72.91	70.27	69.04	66.01
5.00	8.47	38.37	33.87	31.06	37.17	29.09	40.47	43.67	36.24
5.51	9.14	31.85	27.61	13.16	33.74	33.37	35.60	27.75	29.01
6.04	9.75	12.74	10.53	11.08	8.21	17.68	12.83	14.27	12.48
6.59	10.05	9.21	7.62	8.24	5.07	9.51	10.82	9.93	8.63
6.92	10.37	6.74	7.03	8.58	8.35	8.94	8.51	8.04	8.03
7.40	10.12	3.72	4.86	7.13	7.76	7.06	7.14	6.39	6.29
7.93	10.27	3.48	3.87	7.72	6.87	6.94	7.21	7.72	6.26
10.66	10.48	3.17	3.20	5.11	3.74	3.77	4.81	4.47	4.04

Table 3-7 Percent acid adsorbed − Amberlite IRA-96

Initial pH	Final pH	C2 (%)	C3 (%)	IC4 (%)	C4 (%)	IC5 (%)	C5 (%)`	C6 (%)	Total acids (%)
2.76	4.08	73.22	74.92	81.15	80.68	80.12	85.02	89.41	77.80
2.99	4.53	68.74	70.50	73.08	74.62	71.37	81.36	82.58	72.83
3.49	4.82	67.11	73.01	77.83	79.81	85.03	84.36	88.21	75.02
3.95	5.27	66.52	73.23	76.86	78.39	82.54	84.21	88.20	74.60
4.49	5.76	51.83	70.50	72.66	73.15	78.44	79.61	85.51	67.30
5.00	6.25	31.46	48.07	46.52	36.20	49.71	52.29	64.59	43.10
5.51	6.72	14.34	17.72	24.07	24.71	26.81	30.81	37.74	21.15
6.04	7.35	10.01	11.44	20.64	18.20	21.57	28.84	38.97	16.76
6.59	8.31	3.17	4.25	8.58	10.08	11.61	15.62	19.24	7.57
6.92	8.97	3.02	3.91	6.93	9.86	10.05	14.68	20.60	7.31
7.40	9.24	2.57	2.07	2.95	6.21	5.50	7.12	10.15	4.15
7.93	9.31	2.15	2.71	2.03	2.18	3.91	4.55	4.22	2.84
10.66	10.26	2.28	2.94	3.19	3.37	3.69	5.70	8.49	3.61

3.3.4 Effect of equilibrium concentration on adsorption

During the course of the experiment, the initial and final pH of every sample was recorded. The difference between the initial and final solution concentration was assumed to be bound to the ion-exchange resin IRA-67. The percent adsorption capacity achieved for various pH points are shown in Figures 3-3, 3-5, and 3-7. Varying the solution pH, changes the percent adsorption capacity achieved of carboxylic acid. For application in the MixAlco® process, the resin column will adsorb carboxylic acids present in the fermentation broth and the eluted solution will have a higher pH than the solution entering the column; therefore, it is possible to regulate both fermentation pH and acid concentration using an ion-exchange resin column. Additionally, adsorption of carboxylic acids onto resins decreases as the pH increases. This signifies that if the pH of the column can be raised high enough, the adsorbed acids can be regenerated from the column. For maximum efficiency of the MixAlco® process, the fermentation broth must be maintained at near-neutral pH; thus, equilibrium pH is an important factor when considering adsorption efficiency of resins. Plotting the initial solution pH and the equilibrium solution pH in Figures 3-4, 3-6, and 3-8 helps demonstrate the effect of equilibrium pH on adsorption capacity. In conclusion, adsorption of carboxylic acids generally increased with increase in concentration or decreased with increase in pH.

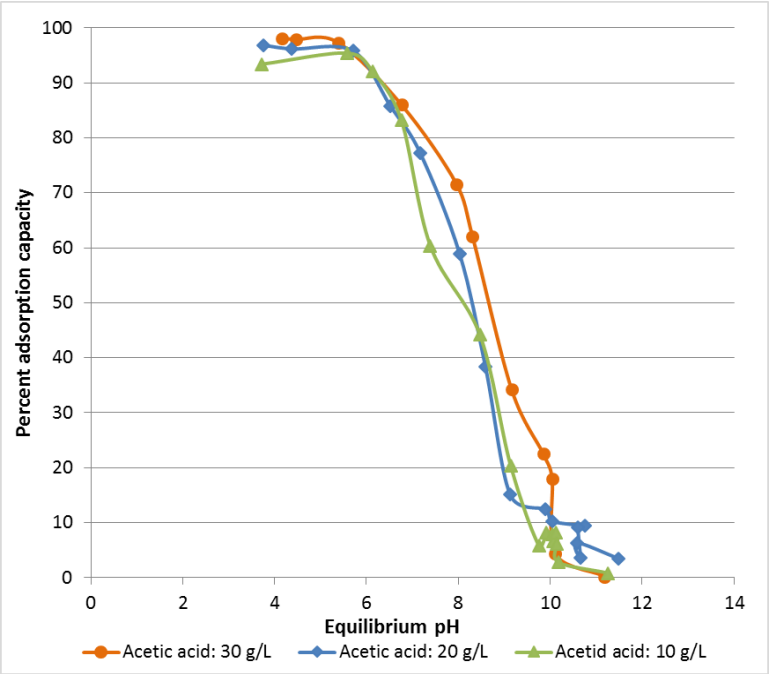


Figure 3-3 Percent adsorption capacities achieved for acetic acid**.**

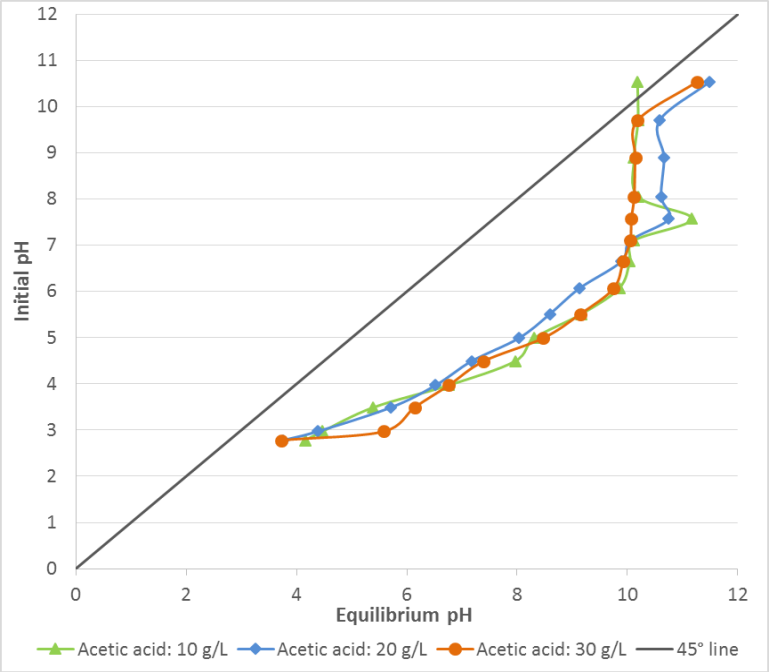


Figure 3-4 Initial and equilibrium pH values for acetic acid samples.

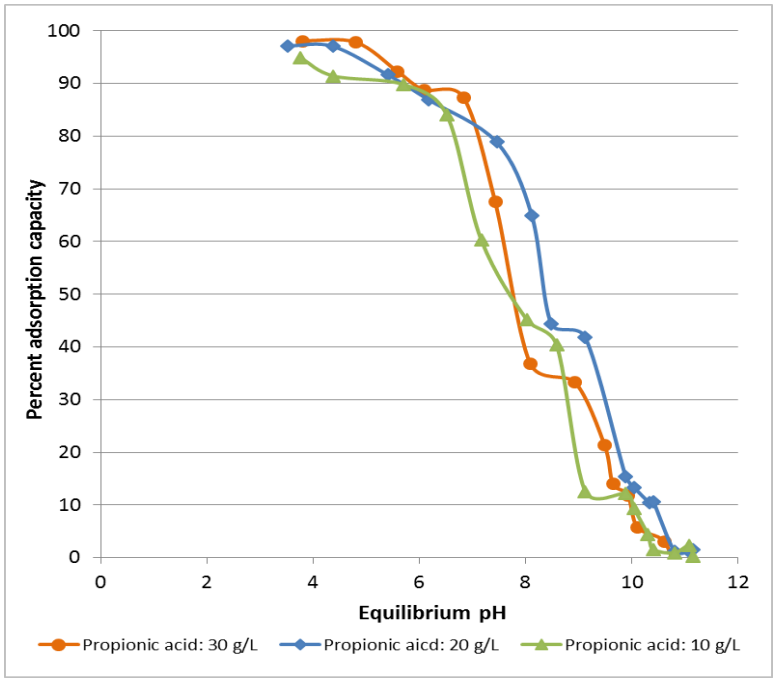


Figure 3-5 Percent adsorption capacities achieved for propionic acid**.**

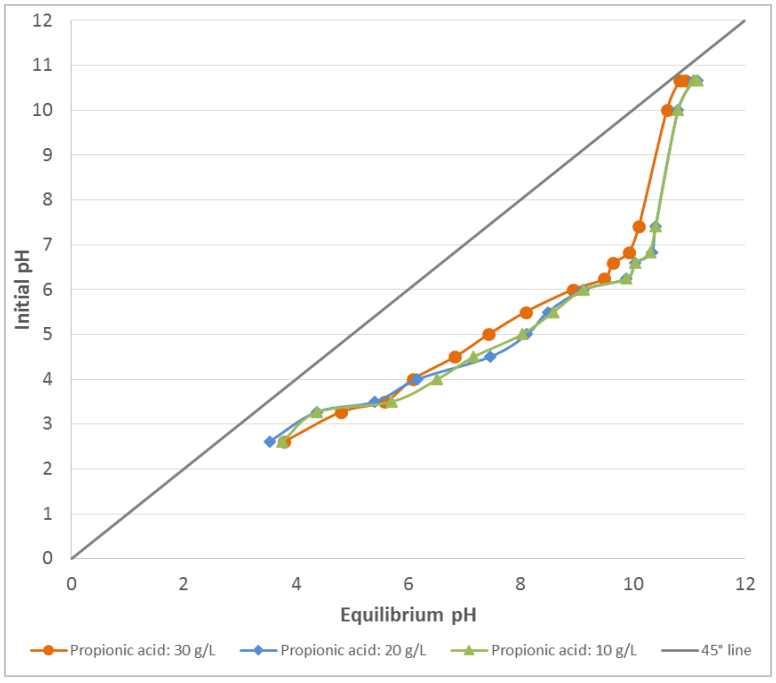


Figure 3- 6 Initial and equilibrium pH values for propionic acid samples.

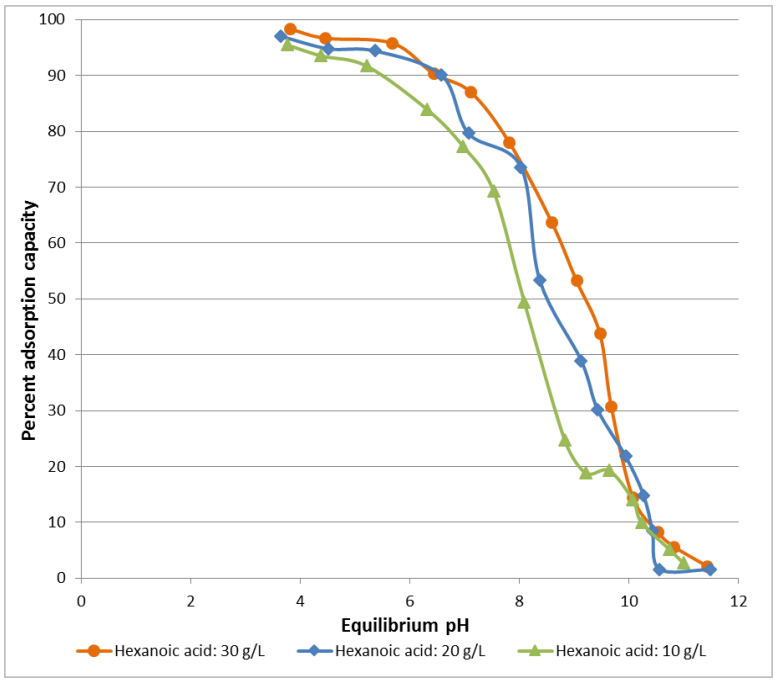


Figure 3- 7 Percent adsorption capacities achieved for hexanoic acid.

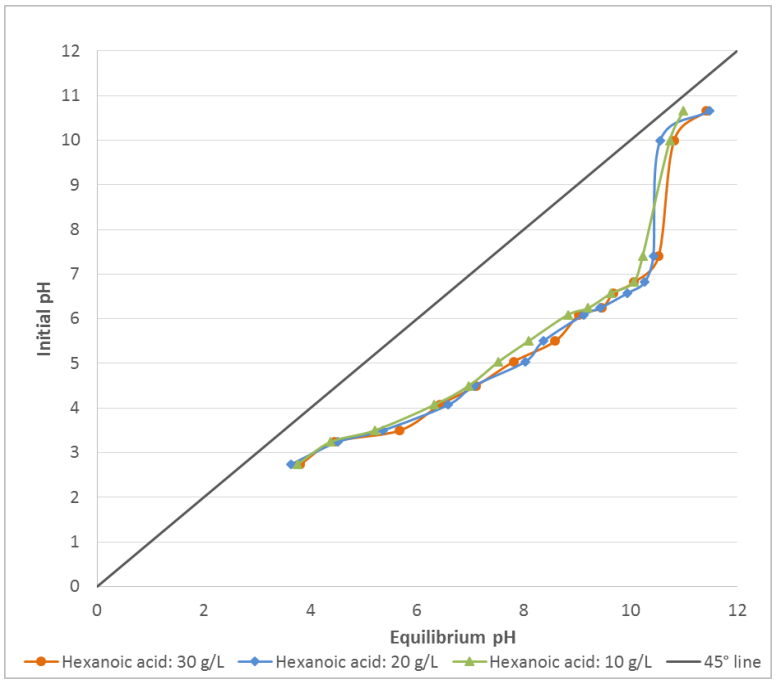


Figure 3- 8 Initial and equilibrium pH values for hexanoic acid

3.3.5 Ion-exchange resins in fixed-bed column operation

Acetic, propionic, and hexanoic acid solutions were passed through a resin column. The initial concentration of the solutions follow: 17.97 g/L (acetic acid), 17.92 g/L (propionic acid), and 17.96 g/L (hexanoic acid). Acid adsorbed on the resin was extracted by passing a solution of NaOH of known concentration and volume through the column. The mass of wet ion-exchange resin in the column was 30 g. It was observed that the resins with adsorbed acids were swollen; upon passage of base, the resins shrunk in size as the acids were eluted. The acid concentration of the solution eluted from the resin column was 2–3 times higher than that of the solution initially passed. This is beneficial for the MixAlco® process because it helps concentrate acid solutions prior to downstream processing. Figure 3-9 shows the adsorption characteristics of Amberlite IRA-67 in column mode by measuring the final concentration of acid solution eluted from the column. Figure 3-10 shows the elution characteristics of Amberlite IRA-67 in column mode by measuring the concentration of acid in the solution eluted when NaOH is passed through the resin column.

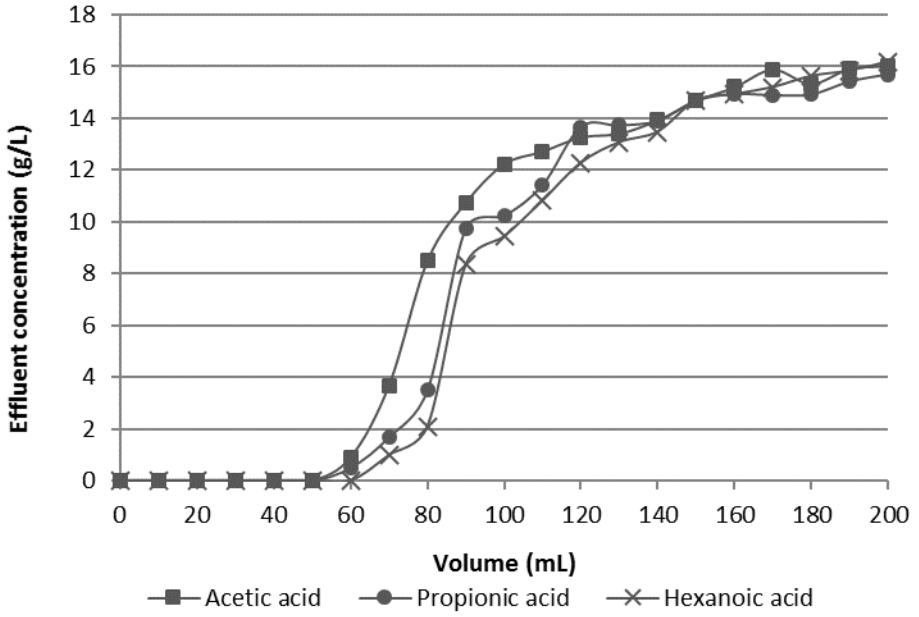


Figure 3- 9 Acid concentration in effluent.

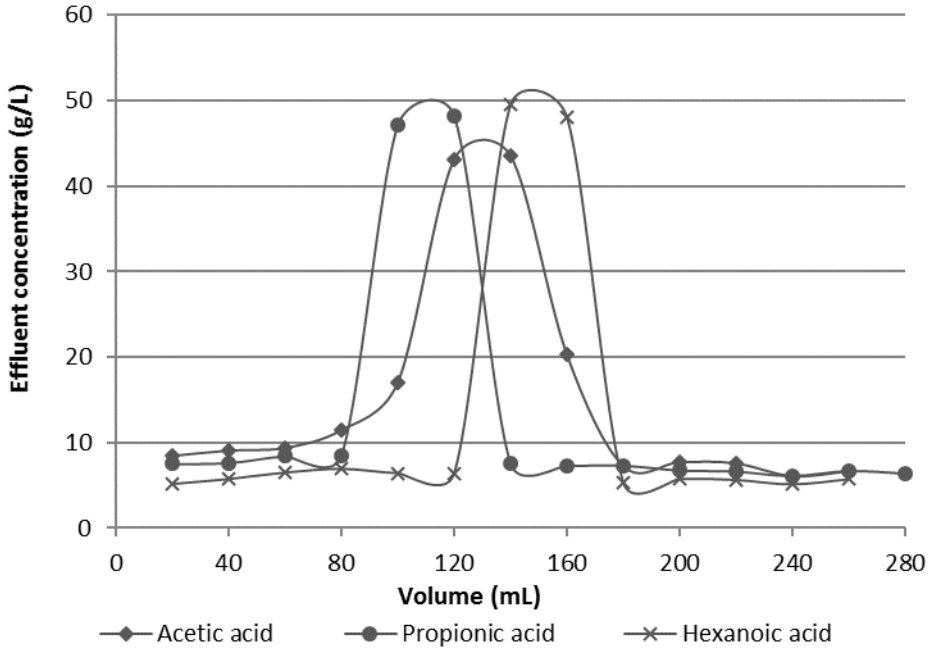


Figure 3- 10 Acid concentration in eluted solution

3.4 Conclusion

This study indicates that ion-exchange resins are a promising method for recovering acids from the MixAlco® process. Amberlite IRA-67 exhibited high adsorption capacity, efficient regeneration characteristics, and preferential adsorption of high-molecular-weight acids. The preferential adsorption of heavier acids is of particular importance in the MixAlco® process. In addition to decreasing carboxylate salt content and reducing inhibition in the fermentors, removing high-molecular-weight acids from the system also creates end products of higher energy value. High-molecular-weight acids have more energy content than low-molecular-weight acids, so they are readily converted to fuels. Additionally, adsorption of carboxylic acids generally increases with increase in concentration or decreases with increase in pH. Ion-exchange resins showed high efficiency in column mode operations. Regeneration studies indicated that more than 85% of adsorbed acid could be recovered from the resin column. The acid concentration of the solution eluted from the resin column was 2–3 times higher than that of the solution initially passed. Thus, ion-exchange resins could be reused for adsorption purposes, making them an efficient and economically viable option.

CHAPTER IV

BATCH FERMENTATIONS USING ION-EXCHANGE RESINS FOR EXTRACTION

4.1 Overview

In the MixAlco® process, a mixed culture of microorganisms ferments biomass to produce carboxylic acids. The advantage of using a mixed culture of microorganisms is that it provides metabolic flexibility and can employ various substrates [9]. As discussed earlier, anaerobic digestion in the fermentor occurs in four stages. However, in the carboxylate platform where the desired products are long-chain acids, production of methane is an impediment for fermentations[35]. Long-chain carboxylic acids are formed from acetic acid by the following disproportionation reactions [36]:

Propionic acid: $7\ HOAc \rightarrow 4\ HOPr + 2\ CO_2 + 2\ H_2O$ (4-1)

Butyric acid: $5\ HOAc \rightarrow 2\ HOBu + 2\ CO_2 + 2\ H_2O$ (4-2)

Valeric acid: $13\ HOAc \rightarrow 4\ HOVa + 7\ CO_2 + 6\ H_2O$ (4.3)

Caproic acid: $4\ HOAc \rightarrow HOCa + 2\ CO_2 + 2\ H_2O$ (4-4)

Heptanoic acid: $19\ HOAc \rightarrow 4\ HOHe + 10\ CO_2 + 10\ H_2O$ (4-5)

Thus, inhibiting methane increases formation of acetic acid, which in turn produces long-chain carboxylic acids. Furthermore, if long-chain carboxylic acids are periodically

removed from the fermentor, it seems reasonable that more acetic acid will combine to form long-chained carboxylic acids.

Increasing the acid concentration reduces alkalinity and thereby decreases pH. The inhibitory effect of high acid concentration and its effect on the microbial community in a bioreactor is considered in the Continuum Particle Distribution Modeling (CPDM) method for the MixAlco® process. According to this method, a *continuum particle* is defined as a collection of particles with a volatile solids mass of one gram upon entering the fermentation. During fermentation, a mixture of acids are produced. The concentration of each acid can be expressed as acetic acid equivalents (Aceq), defined as the reducing potential of equivalent amount of acetic acid [7, 36].

Aceq (mol/L) = acetic (mol/L) + 1.75 × propionic (mol/L) + 2.5 × butyric (mol/L) + 3.25 × valeric (mol/L) + 4.0 × caproic (mol/L) + 4.75 × heptanoic (mol/L) (4-6)

Aceq (g/L) = Aceq (mol/L) × 60.05 (g/mol) (4-7)

The advantage of using acetic acid equivalents is that the various carboxylic acid fractions can be represented as a single concentration. As the fermentation reaction proceeds and biomass gets digested, its reactivity reduces and rate of production of carboxylic acids decreases. The following equation describes the effect of conversion and product concentration on the reaction rate *r* (Eq. 4-8) [36]:

$$r = \frac{\varepsilon(1-x)^f}{1+g(\phi \times \text{Aceq})^h} \quad (4\text{-}8)$$

where x represents volatile solids conversion, ϕ is the ratio of total acids to acetic acid equivalents, e,f,g,and h are constants that can be determined by 'solver' in *Excel* using least square analysis of specific rate and reaction rate r [36].

The numerator in Eq. 4-8 represents the impact of declining substrate reactivity with increasing conversion x. The denominator, expressed in acetic acid equivalents, represents the inhibitory effect on the microorganisms at high product concentrations. As substrate is consumed, the numerator decreases, thus slowing the reaction. Further, increase in product concentration increases the denominator thus decreasing the reaction. Holtzapple *et al.* [1] stated that when the pH of the fermentation broth was maintained between 5.8 and 6.2, the exponent on the product inhibition term was 4.38. However, for fermentations conducted at near-neutral pH, the product inhibition exponent was reduced to 2.11. Thus, pH and acid concentration are two important parameters that affect fermentation conversion and yield.

In anaerobic digestors or animal guts, acetate is either continuously removed by methanogens or is assimilated by the animal; thus, acid concentration is low. Anion-exchange resins can be used to maintain low acid concentration in fermentors by extracting carboxylic acids as they are produced, analogous to how acids are removed in animal guts.

In this study, a weak-base anion-exchange resin (Amberlite® IRA-67) was used to adsorb carboxylic acid from fermentation broth. The experiment was designed to compare a control fermentor (acids accumulate under controlled pH using $MgCO_3$ buffer) to a fermentor that uses ion-exchange resins to remove product and regulate pH. Three

different substrates were chosen to check the variability in fermentation parameters (conversion, yield, and selectivity) with different biomass feedstock.

4.2 Experimental methods

4.2.1 Fermentation

Three different batch fermentation sets were prepared using α-cellulose powder, shredded office paper, and lime-pretreated corn stover as substrates. Each fermentation set had one $MgCO_3$ control fermentor and two ion-exchange resin fermentors [8]. Table 4-1 describes the experimental design for α-cellulose, office paper, and lime-pretreated corn stover fermentations. Fermentors were kept in a rotating incubator maintained at 40ºC. Biogas − a mixture of hydrogen (H_2), carbon dioxide (CO_2), and methane (CH_4) − was formed as the fermentation proceeded. During the experiment, each fermentor was vented every 24 h for the first 10 days and every 48 h thereafter to remove biogas and thereby prevent fermentor rupture. Then, the fermentor stoppers were removed and the initial pH of the fermentation broth was measured. Next, the fermentors were centrifuged at 4000 rpm for 25 min to separate liquid and solid fractions. A sample (1 mL) of the liquid fraction was collected to analyze the acid concentration. In the control fermentor, if the initial pH for the fermentation broth was less than 6.8, a measured quantity of magnesium carbonate ($MgCO_3$) was added until the pH was near neutrality. After adding $MgCO_3$, the final pH was noted. In the case of the ion-exchange (IR) duplicates, if the pH was less than 6.8, all of the fermentation broth was passed through a column containing Amberlite® IRA-67 ion-exchange resins. The fermentation broth leaving the column was reintroduced to the column until its exit pH was in the range of 6.8−7.2. After the

fermentation broth passed through the resin column, the final pH was recorded and a 1-mL sample of fermentation broth was collected for acid concentration analysis. However, during the initial few days, the pH of some fermentors was above 7.2. In such situations, no liquid was passed through the ion exchange resin column but instead, CO_2 was bubbled into the fermentors produce carbonic acid, which lowered the pH to near neutrality.

$$CO_2 + H_2O \leftrightarrow H_2CO_3 \quad (4\text{-}7)$$

Carbonic acid further dissociates into bicarbonate and carbonate ions.

$$H_2CO_3 \leftrightarrow HCO_3^- + H^+ \quad (4\text{-}8)$$

$$HCO_3^- \leftrightarrow CO_3^{2-} + H^+ \quad (4\text{-}9)$$

However, adding CO_2 to the system does not accumulate carbonate or bicarbonate because when carboxylic acids are produced, inorganic carbon eventually leaves the fermentor as carbon dioxide. While passing the fermentation broth through the ion exchange resins, bicarbonate from the dissolved CO_2 would compete with the carboxylate anions for adsorption in resins. However, no study was undertaken to understand the selectivity of adsorption of bicarbonate and carboxylate anions. After adjusting the pH of the fermentors, they were purged with nitrogen gas from a high-pressure liquid nitrogen cylinder to maintain anaerobic conditions, and were placed back into the incubator.

Acid concentrations and pH of all fermentors were measured every 48 h. A liquid sample (0.5 mL) was collected from the fermentors before monitoring. Gas chromatography (GC) samples were prepared and the concentration of acetic, propionic, butyric, valeric, caproic, and heptanoic acids in the fermentation broth was analyzed by a

gas chromatograph (GC) system equipped with a flame ionization detector (FID) and an Agilent 7683 automatic liquid sampler.

4.2.2 Ion-exchange column operation

The difference between the initial and final acid concentration quantified the amount of carboxylic acid adsorbed onto the resin column. The carboxylic acid concentration measured using the gas chromatograph (GC) was reported in g/L. This value was converted to the mass of carboxylic acid adsorbed onto the resin by using the following equation:

$$\text{Acid adsorbed (g)} = (C_{\text{initial}} \times V_i) - (C_{\text{final}} \times V_f) \quad (4\text{-}10)$$

where C_{initial} and C_{final} are acid concentrations expressed in (g/L), V_i is the initial volume of fermentation broth passed through the resin column expressed in (L), and V_f is the volume of fermentation broth collected after it passed through the resin column expressed in (L).

Table 4-1 Experimental design for fermentations

Sr. No.	**Feed**		**Total solid loading (g/L)**	**Inocula (mL)**	**Deoxygenated water (mL)**	**Iodoform$_{initial}$ (µL)**	**Urea (mg)**
	Nutrient (20%)	**Biomass (80%)**					
1	Chicken manure	α-cellulose	100	50	348	120	0.8
2	Chicken manure	Office paper	100	50	348	120	0.8
3	Chicken manure	Lime-pretreated corn stover	100	50	348	120	0.8

A solution of known volume and concentration of NaOH was prepared and passed through the ion-exchange resin columns loaded with carboxylic acids. The equivalent amount of sodium hydroxide (NaOH) required to wash the resins for complete recovery of carboxylic acids was calculated for a given mass of acid adsorbed. The volume of NaOH solution to be passed was chosen so that it was greater than the volume of liquid in the resins and its concentration was chosen to provide required equivalents of NaOH. The eluted solution was collected and its volume was noted. A sample (0.5 mL) was collected and used to prepare GC samples to determine the acid concentration. To find the mass of carboxylic acids recovered, the resulting total acid concentration for each sample was multiplied by the volume of solution eluted. This value was compared to the mass of carboxylic acid adsorbed onto the resin for each sample and if the difference in acid concentration was more than 20%, the NaOH solution was passed through the column again.

The fermentation broth passed into the column had a higher carboxylic acid concentration than that flowing out of the column; therefore, the pH of the outgoing stream was higher than the inlet. During the course of the experiment, the pH measurements served as monitoring criteria. Every 48 h, the pH of the fermentation broth was adjusted to 6.8–7.2 by adding CO_2 to lower the pH or passing fermentation broth through ion exchange resin column to increase the pH. This range was chosen because microbial activity was most efficient in the given pH range.

4.3 Results and discussion

4.3.1 Biogas analysis

During the first 22 days of the fermentation, biogas production increased and steadily declined thereafter. For the first 10 days of the fermentation, iodoform was added to the fermentor every 24 h and thereafter was added every 4 days. Iodoform inhibited methane formation in the fermentors; as shown by biogas analysis that did not detect methane in the headspace gases of the fermentors. Sample biogas analysis charts are shown in Appendix A.

4.3.2 Acid concentrations

Figures 4-1, 4-2, and 4-3 show the total carboxylic acid and Aceq concentrations for fermentations performed using α-cellulose, shredded office paper, and lime-pretreated corn stover. The daily carboxylic acid concentrations and total acid production varied with substrate. Table 4-4 summarizes the final total acid concentration in each fermentor. For IR fermentors, the table shows average concentration measured in the replicate experiment. Comparing the acid concentration profiles of the $MgCO_3$ control and IR shows that extracting acids reduced the acid concentration in the fermentors. In the $MgCO_3$ control, the acid concentration changed rapidly during the initial days of the fermentation run but changed more slowly at the end.

Table 4- 2 Final total acid concentration in fermentor.

Parameter	α- cellulose		Office paper		Lime-pretreated corn stover	
	Control	IR	Control	IR	Control	IR
Total acid concentration (g/L)	17.96	12.84±1.13	31.81	21.90±1.49	30.05	10.59±0.85
Acetic acid equivalents (g/L)	19.14	16.35±1.02	50.29	31.71±0.97	44.84	14.88±1.92

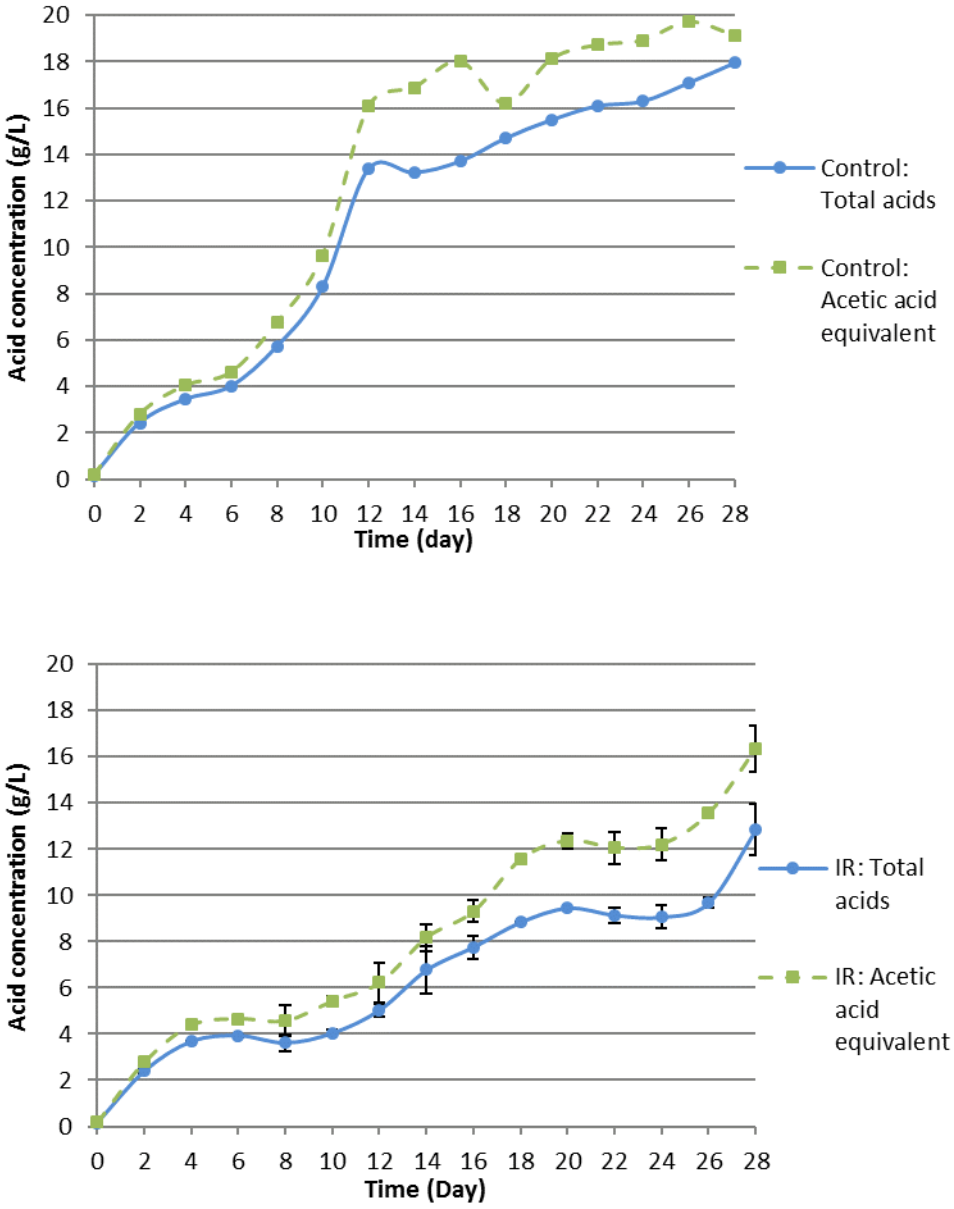


Figure 4-1 Acid concentration in fermentor for α-cellulose.

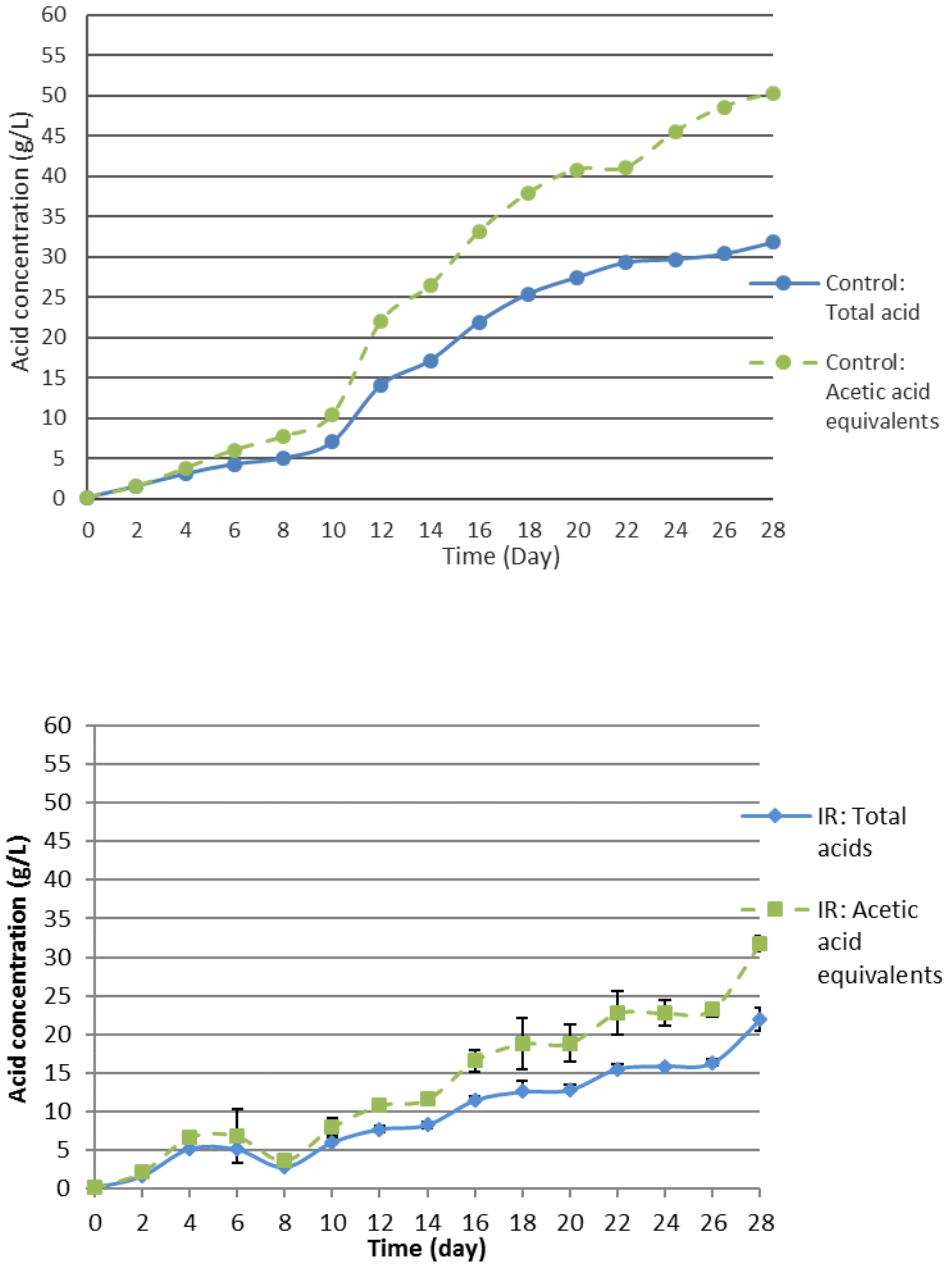


Figure 4-2 Acid concentration in fermentor for office paper.

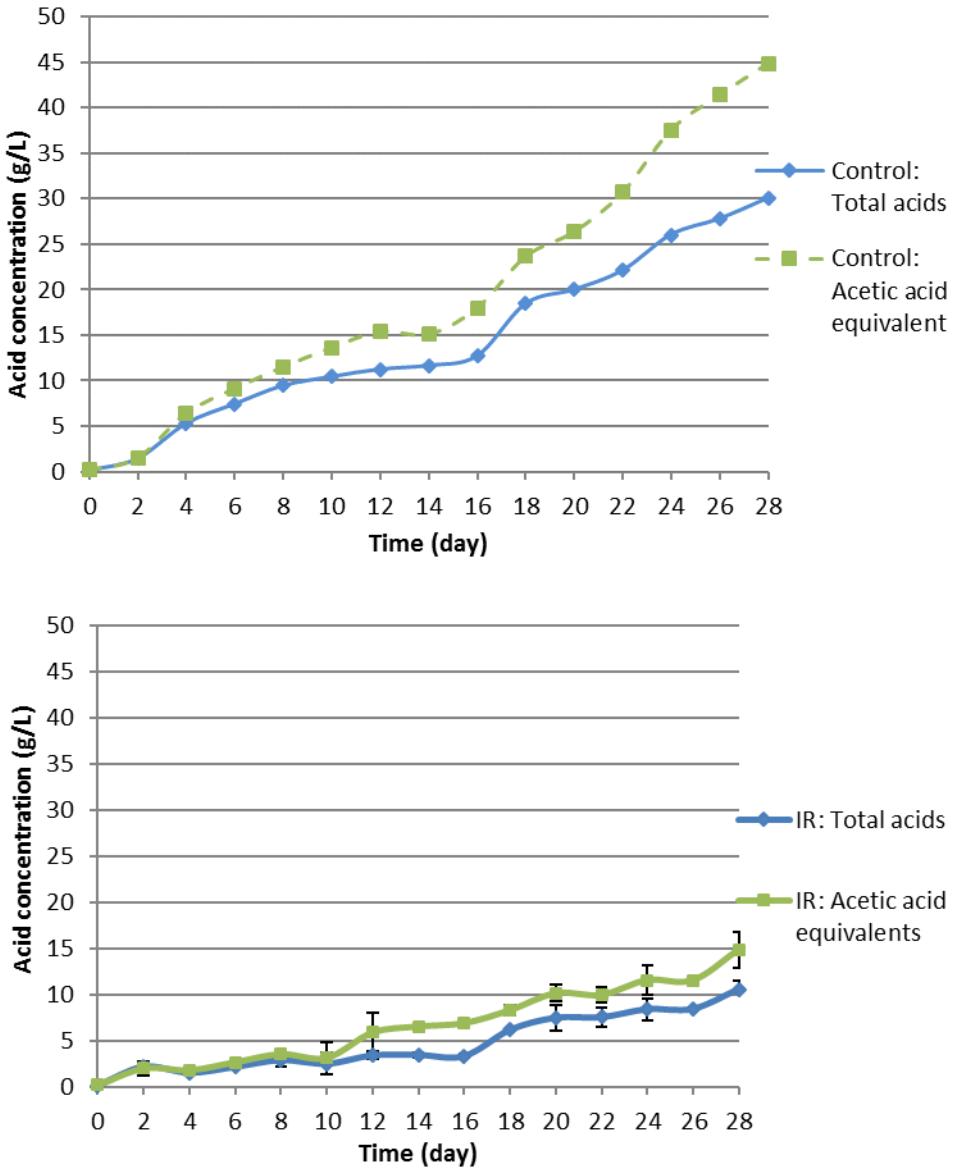


Figure 4-3 Acid concentration in fermentor for lime-pretreated corn stover.

During the course of the experiment, fermentation broth for IR (ion exchange resin) fermentors was periodically passed through the ion-exchange resin column, which adsorbed carboxylic acids and helped maintain fermentation pH in the desirable range. In the IR systems, measurements were made of the amount of acids in the fermentor and the amount of acids adsorbed on the resins. *Total acids produced* is defined as the sum of the acids recovered by the ion-exchange resins and the acids in the fermentor. For each substrate, Figures 4-4, 4-6, and 4-8 depict the yield based on total carboxylic acid produced in the $MgCO_3$ control fermentor, in the IR fermentors, and in the IR system (broth +resin), for the three different substrates. Figures 4-5, 4-7, and 4-9 depict the Aceq. yield in the $MgCO_3$ control fermentor, Aceq. yield based on total carboxylic acid produced in the same systems. Figures 4-10, 4-11, and 4-12 compare carboxylic acid distribution in $MgCO_3$ control and IR for different substrate types during the last 10 days of the fermentation. In the $MgCO_3$ control, acetic acid is greater than the other carboxylic acid fractions. In the IR system, the dominance of acetic acid is reduced and there is a greater fraction of heavier carboxylic acids (C4–C8).

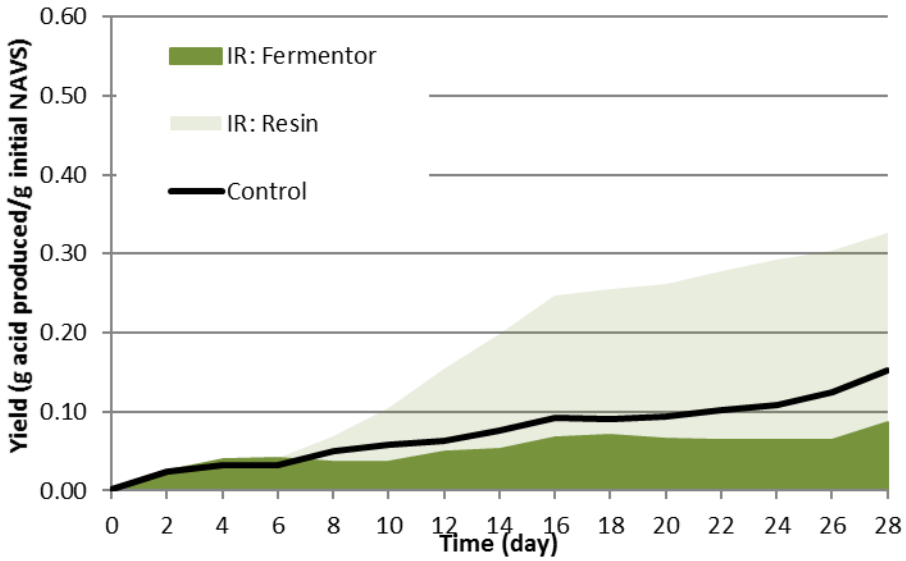


Figure 4-4 Yield: α-cellulose.

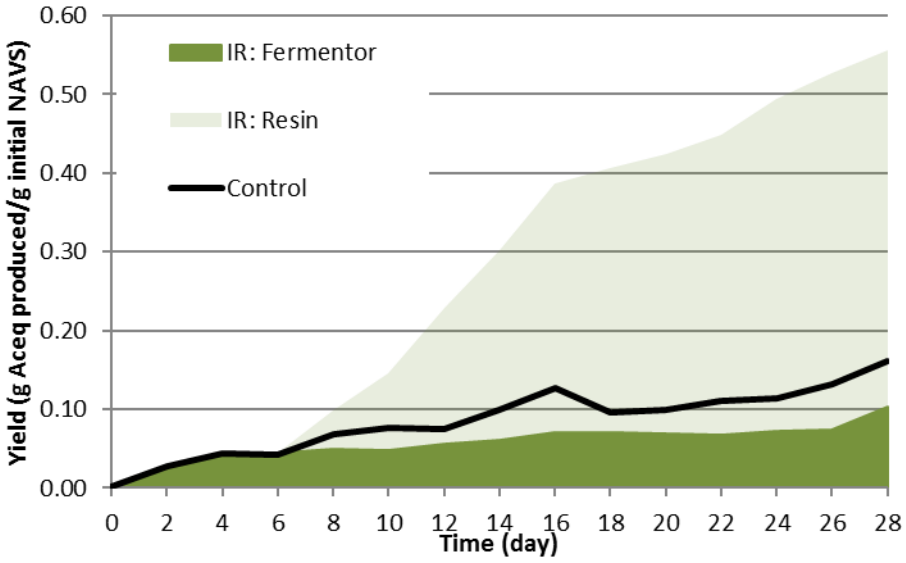


Figure 4-5 Yield (Aceq): α-cellulose.

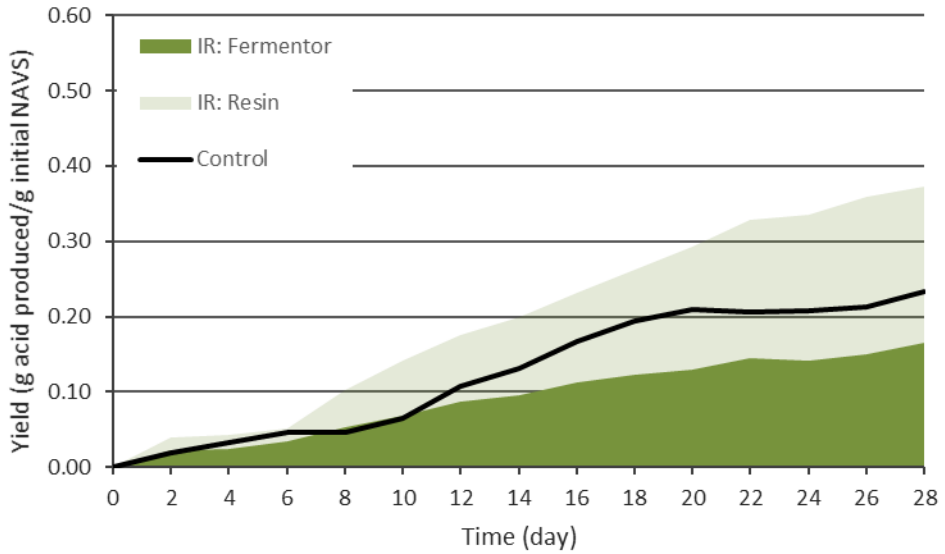


Figure 4-6 Yield: office paper.

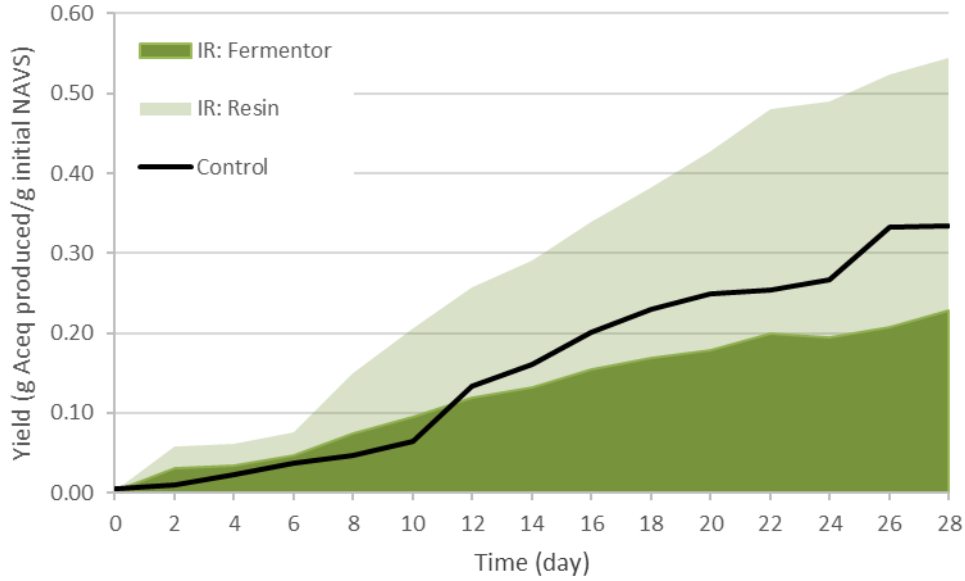


Figure 4-7 Yield (Aceq): office paper.

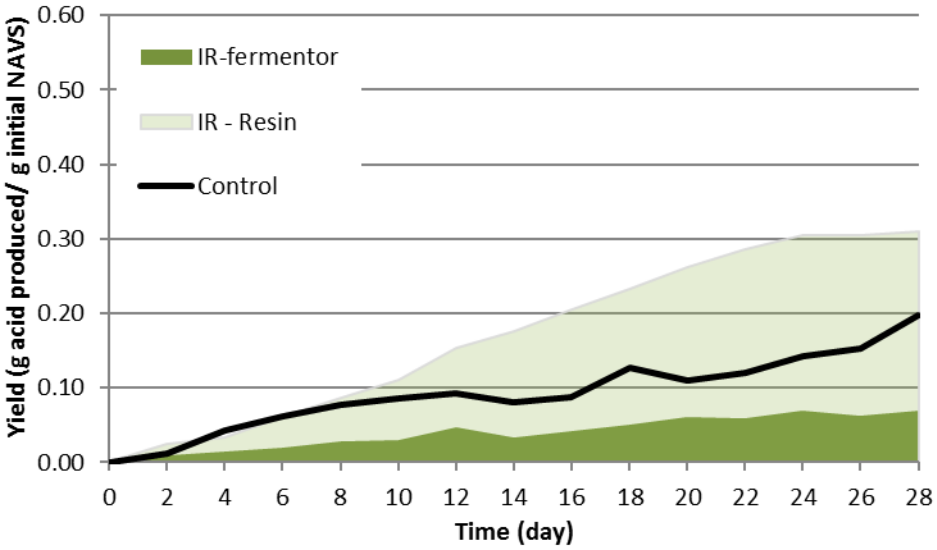


Figure 4-8 Yield: lime-pretreated corn stover.

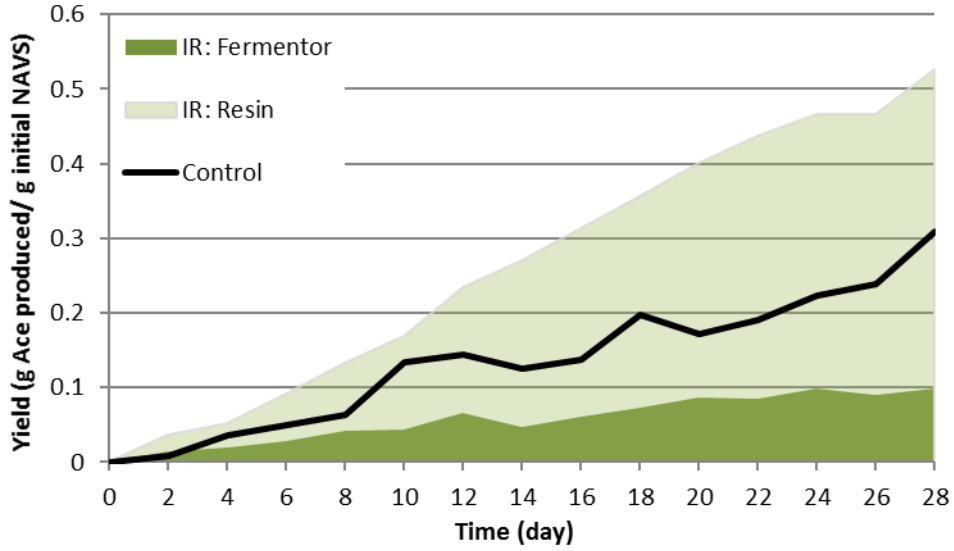


Figure 4-9 Yield (Aceq): lime-pretreated corn stover.

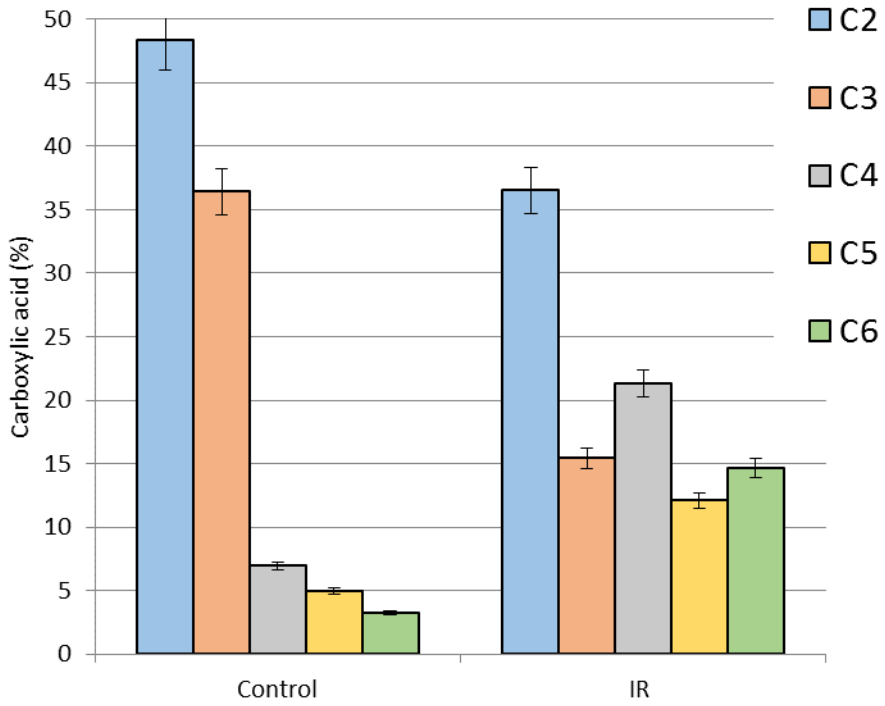


Figure 4-10 Acid distribution: α-cellulose.

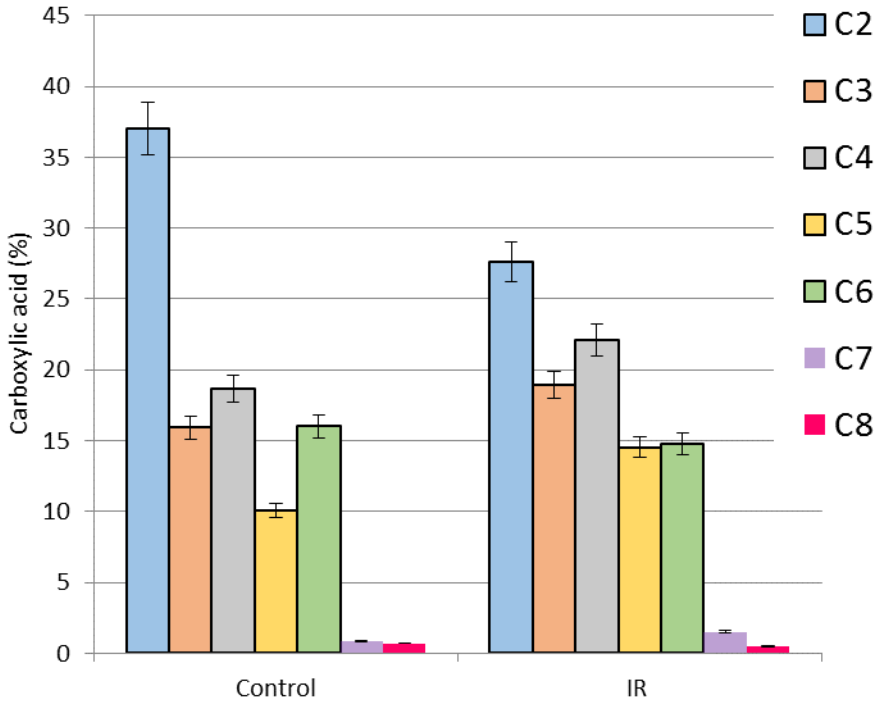


Figure 4-11 Acid distribution: office paper.

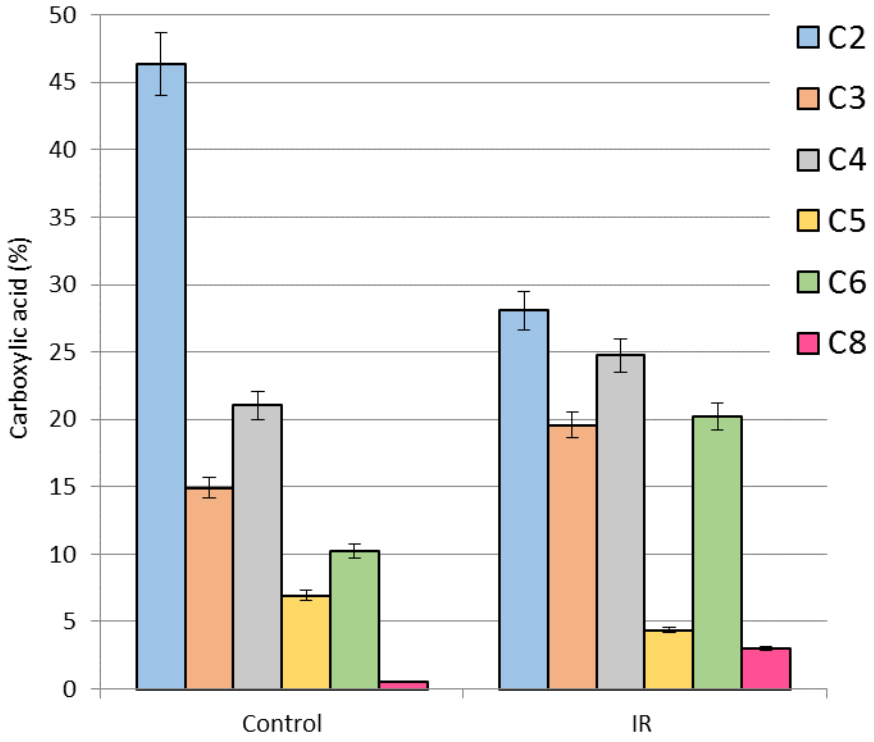


Figure 4-12 Acid distribution: lime-pretreated corn stover.

4.3.3 Regeneration

When completely saturated with carboxylic acids, the ion-exchange resins had to be eluted with a base to recover carboxylic acids. The NaOH solution was passed through the resin column and was collected from the column outlet. Acid concentration analysis of the eluted solution showed that more than 75% of the acid on the resins were regenerated. The acid concentration of the solution eluted from the resin column was 2–3 times higher than that of the solution initially passed. Figures 4-13, 4-14, and 4-15 show the quantity of acid initially adsorbed and later recovered from the resins. For calculating yield and selectivity, total acid adsorbed onto the resin (g) was used instead of the total acid recovered from column (g) after passing NaOH solution.

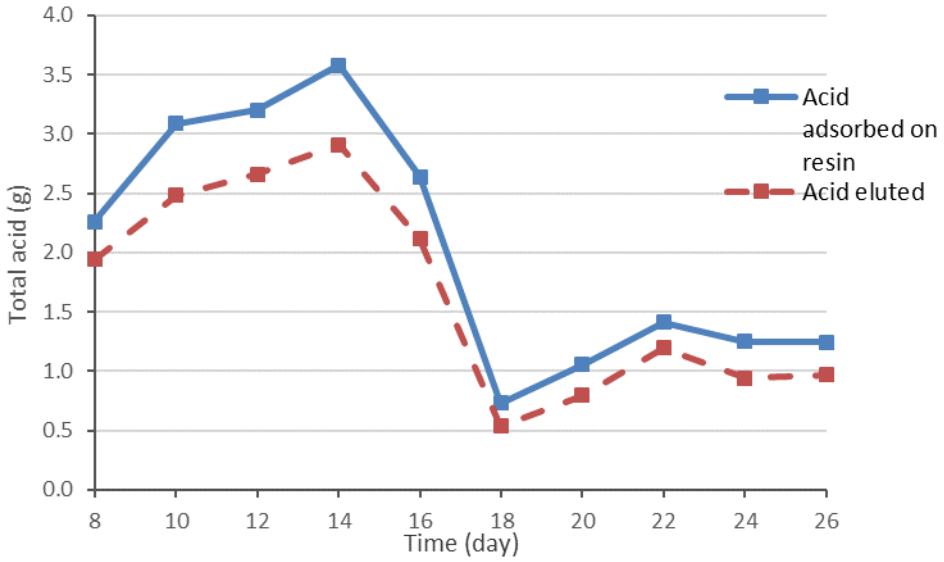


Figure 4-13 Acid adsorption and recovery: α-cellulose.

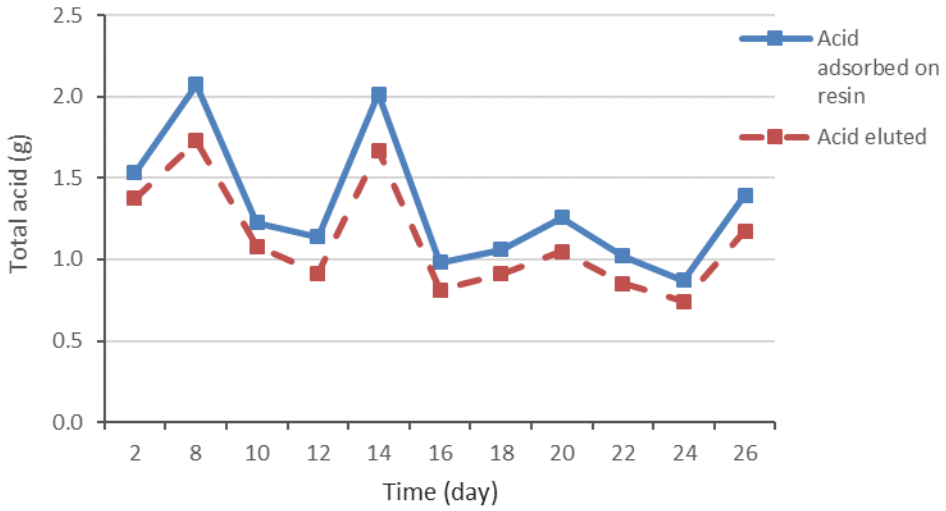


Figure 4-14 Acid adsorption and recovery: office paper.

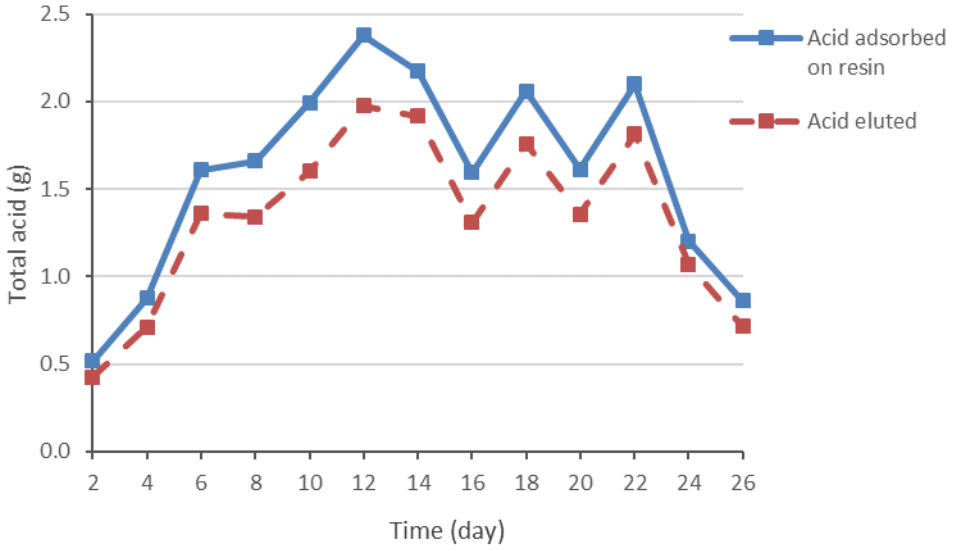


Figure 4-15 Acid adsorption and recovery: lime-pretreated corn stover.

4.3.4 Fermentation parameters

The fermentation performance was measured by parameters such as conversion, yield, and selectivity. The results are summarized in Table 4-3.

Conversion, as described in Section 2.3, measures NAVS digested (g) with respect to NAVS fed (g) into the system. For all the three substrates used in this study, the conversion for IR was higher than that of the $MgCO_3$ control. Reducing inhibition by IR extraction increased conversion of all three substrates.

Yield quantifies the mass of total acid produced in the fermentor (including the acid adsorbed by the resin) per mass of NAVS fed. As described in Section 2.3, , the yield was also calculated using the total acid produced expressed in terms of acetic acid equivalents. For all three substrates, the yield and $yield_{aceq}$ were higher for IR than for the $MgCO_3$ control; thus, reducing product inhibition increases total acid production.

Mixed-acid fermentations have a wide range of products such as H_2, CH_4, CO_2, lactate, acetate, butyrate, propionate, caproate, ethanol, etc. However, for the MixAlco® process, the most valuable products are carboxylic acid. *Selectivity* measures fermentation performance with respect to formation of desired products and is defined as the mass of total carboxylic acid produced (g) per mass of NAVS digested (g). As described in Section 2.3, the acetic acid equivalents of the carboxylic acids produced was calculated and used to calculate the Aceq selectivity. Theoretically, complete fermentation of 1 g of cellulose would produce 1.11 g of *acetic* acid and this can be defined as the *maximum theoretical selectivity*, which is shown in Figure 4-18. Among all the substrates, the difference between selectivity of the $MgCO_3$ control and IR was highest for α-cellulose.

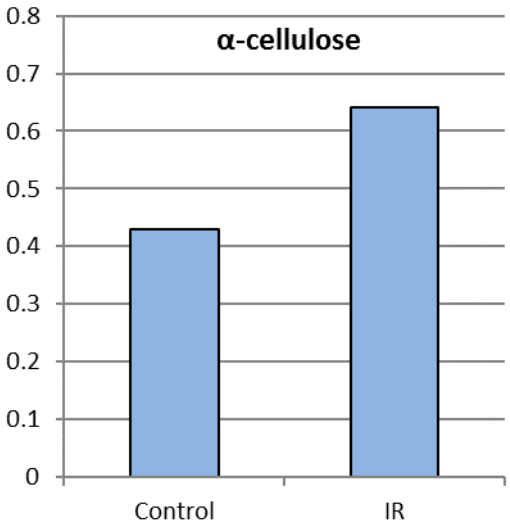
The results of this study demonstrate that removing inhibition caused by high concentrations of carboxylic acids increases the fermentation rate and product formation. Figures 4-16, 4-17, and 4-18 compare fermentation performance measures of $MgCO_3$ control and IR for α-cellulose, office paper, and lime-pretreated corn stover, respectively. Biogas analysis shows that no methane was produced by any system. However, succinic acid was produced in IR fermentors from Days 18–24. It is possible that succinic or lactic acid is being formed during the fermentation and future studies should measure these components to determine their impact on selectivity.

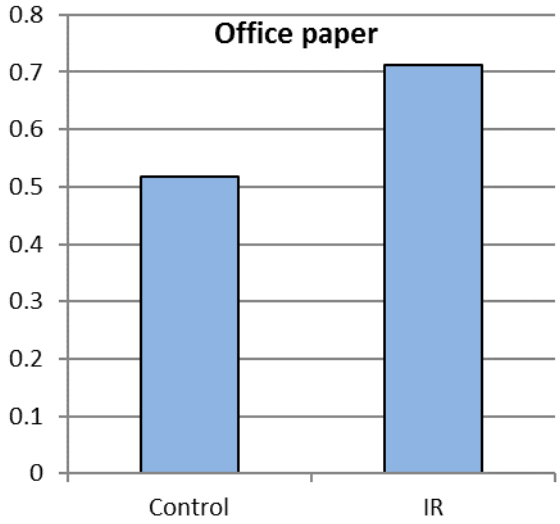
4.4 Conclusions

Extraction of carboxylic acid benefited the fermentations. Although acid production varied for all three substrates, total acid production for IR systems was much greater than that of $MgCO_3$ controls. Periodic extraction of carboxylic acids from the IR system allowed the acid concentration in the α-cellulose and lime-pretreated corn stover fermentors to be 4–10 g/L and in the paper fermentor acid concentration was to be 7–15 g/L. Although the acid concentrations in the IR fermentors were lower than that in the control fermentors, the total acid produced by the IR system was much higher than that of the control fermentors because acids were extracted by the resins. This indicates that it is possible to run fermentations at low acid concentrations, which reduce product inhibition and increase yield. It was interesting to note that the IR systems had a higher fraction of long-chain carboxylic acids than those with $MgCO_3$ control. To represent the fractions as a single concentration, mixed acids produced were converted to acetic acid equivalents thereby providing a common platform for comparison.

Table 4- 3 Fermentation performance measures for batch fermentations

Parameter	α-cellulose		Office paper		Lime-pretreated corn stover	
	Control	IR	Control	IR	Control	IR
Acid in fermentor (g)	5.73	3.290.16	7.77	5.4±0.27	7.39	2.67±0.13
Acid adsorbed on resin (g)	–	10.18±0.51	–	6.83±0.34	–	8.73±0.44
Total acid in system (g)	5.73	10.18±0.51	7.77	12.23±0.61	7.39	11.39±0.5695
Total Aceq in system (g)	6.1	20.98±1.04	10.96	17.81±0.89	11.02	17.42±0.87
Carboxylic acid concentration in fermentor (g/L)	17.96	12.84±1.13	31.81	21.90±1.49	30.05	10.59±0.85
Acetic acid equivalents (g/L)	19.13	16.35±1.02	44.93	31.71±0.97	44.84	14.88±1.92
Conversion (g NAVS digested/g NAVS fed)	0.43	0.64±0.03	0.5	0.71±0.03	0.49	0.65±0.03
Yield (g total carboxylic acid produced/ g NAVS fed)	0.15	0.35±0.01	0.24	0.37±0.01	0.2	0.35±0.01
Aceq yield (g Aceq produced/ g NAVS fed)	0.16	0.55±0.02	0.33	0.54±0.03	0.3	0.53±0.02
Selectivity (g total carboxylic acid produced/g NAVS digested)	0.35	0.55±0.03	0.47	0.52±0.02	0.41	0.53±0.02
Aceq selectivity (g Aceq produced/ g NAVS digested)	0.38	0.86±0.04	0.53	0.76±0.03	0.61	0.80±0.04





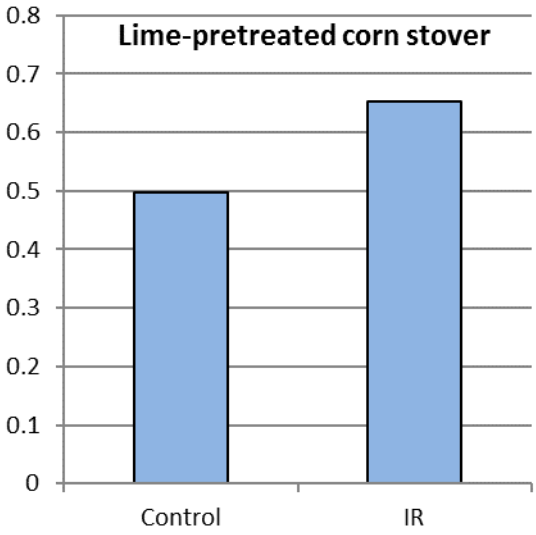


Figure 4-16 Conversion comparison

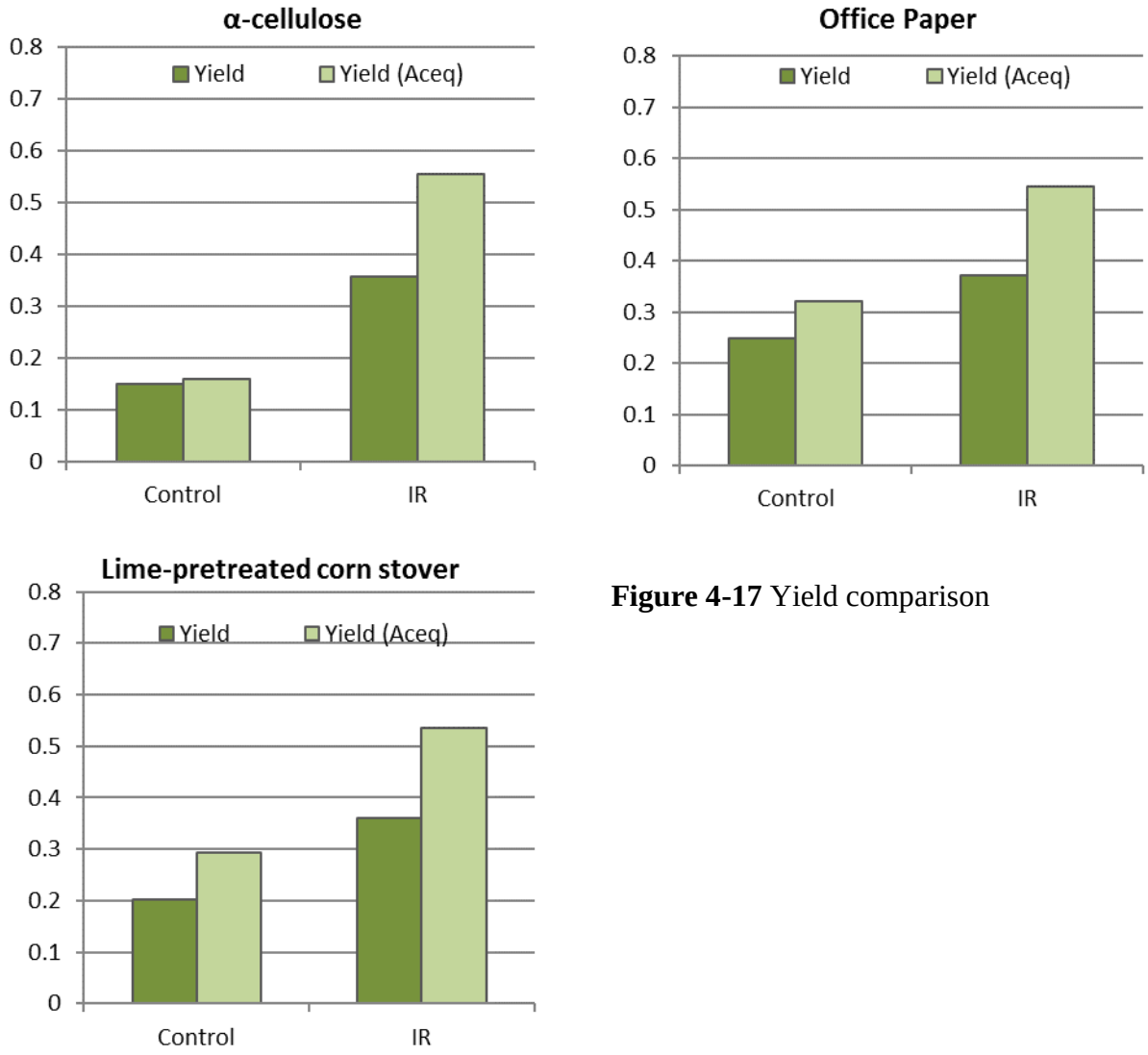
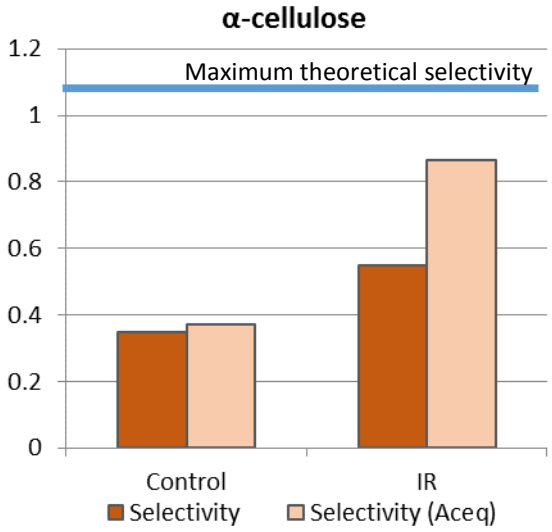


Figure 4-17 Yield comparison



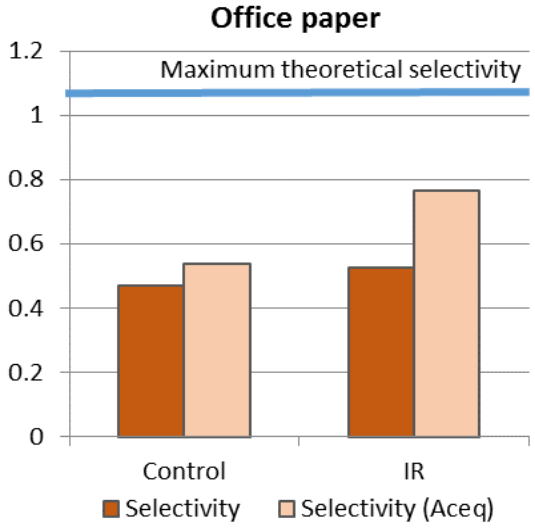


Figure 4-18 Selectivity comparison.

The Aceq selectivity calculated for α-cellulose powder, office paper, and lime-pretreated corn stover approaches the theoretical Aceq selectivity of each system. The efficient adsorption and ease of desorption of acids from Amberlite IRA-67 indicates that this resin can be used industrially. Furthermore, the increased conversion indicates that product inhibition was reduced with periodic extraction of acids. Other fermentation parameters (total acid production, yield, and selectivity) also improved by IR extractions. All fermentations were terminated on Day 28; however, Figures 4-4, 4-5, and 4-6 clearly depict that acid production had not reached a maximum and was still following an increasing trend. If the fermentations had run for longer, there was a likelihood that the conversion and yield would have increased too. Further studies should be devoted to performing fermentations with IR extraction for a longer duration to fully understand how IR extraction increases the efficiency of the system as indicated by increase in parameters such as conversion and yield.

CHAPTER V

CONCLUSION

This work explored the effect of removing carboxylic acids from mixed-culture fermentations. The initial study of ion-exchange resins showed that acid could efficiently be adsorbed and desorbed. Adsorption characteristics of resins were studied in both batch and column operation modes. Adsorption characteristics of Amberlite IRA-67 showed that adsorption of acids increased with increases in acid concentration and decreased with increase in pH. Thus, acid adsorbed on the resin can be eluted by passing a base and increasing the pH of the solution passing through the resin column. Once the acids adsorbed on the resins are eluted, the resin column can be reused, making resins an economically viable option for the MixAlco® process.

Using ion-exchange resins, acid concentrations in fermentors could be kept low which helped reduce product inhibition and increased process yield. While performing adsorption studies, it was noticed that high-molecular-weight acids were adsorbed with more ease than low-molecular-weight acids. This is of particular importance in the MixAlco® process because it allows for selective recovery of long-chain acids, which are more valuable for fuels. Furthermore, the concentration of acid in the eluted solution was at least 2-3 times its influent concentration, which indicates that in the MixAlco® process, acids recovered during elution are pre-concentrated and thereby reduce downstream processing costs.

The IR systems showed higher conversion, yield, and selectivity compared to $MgCO_3$ control fermentors. Conversion improved by a factor of 1.50, 1.42, and 1.33 for α-cellulose, office paper, and lime-pretreated corn stover, respectively. Yield improved by a factor of 2.2, 1.54, and 1.75 for α-cellulose, office paper, and lime-pretreated corn stover, respectively. Selectivity improved by a factor of 1.57, 1.1 and 1.3 for α-cellulose, office paper, and lime-pretreated corn stover, respectively.

The results obtained from this work can be applied to propagated fixed-bed fermentation systems, which are proposed for industrial fermentations [2]. In this system, four fermentors are arranged in a manner such that the fermentor with the most-digested biomass receives fresh liquid which cascades down to the least-digested and exits as product liquid. Through each successive stage, the carboxylate salt concentration in the liquid increases. Ion exchange columns can be fitted into the system such that the liquid passes through the resin column before entering a fermentor. Thus, the fermentors will have less product inhibition because the incoming liquid would have very low carboxylate salt content. Although use of anion exchange resins to remove carboxylate anions from batch fermentations is promising, future studies must be pursued to better understand factors that affect yield, selectivity, and production of high-molecular-weight acids.

REFERENCES

1. Holtzapple, M.T., et al., *Biomass conversion to mixed alcohol fuels using the MixAlco process.* Applied Biochemistry and Biotechnology, 1999. **77-9**: p. 609-631.
2. United States Energy Information Administration (U.S.E.I.A), *International energy outlook 2013*. 2013: Washington DC, 20585.
3. Golub, K. and M.T. Holtzapple, *Effect of bioreactor mode of operation on mixed-acid fermentations*. 2012, Texas A&M University: College Station, Tex. p. 1 online resource.
4. Pimentel, D., et al., *Biofuel impacts on world food supply: Use of fossil fuel, land and water resources.* Energies, 2008. **1**(2): p. 41-78.
5. Schmer, M.R., et al., *Net energy of cellulosic ethanol from switchgrass.* Proceedings of the National Academy of Sciences of the United States of America, 2008. **105**(2): p. 464-469.
6. Sterzinger, G., *Making biomass energy a contender.* Technology Review, 1995. **98**(7): p. 34-40.
7. Holtzapple, M.T. and C.B. Granda, *Carboxylate platform: The MixAlco process part 1: comparison of three biomass conversion platforms.* Applied Biochemistry and Biotechnology, 2009. **156**(1-3): p. 525-536.
8. Agler, M.T., et al., *Waste to bioproduct conversion with undefined mixed cultures: the carboxylate platform.* Trends in Biotechnology, 2011. **29**(2): p. 70-78.

9. Zhang, F., et al., *Fatty acids production from hydrogen and carbon dioxide by mixed culture in the membrane biofilm reactor.* Water Research, 2013. **47**(16): p. 6122-6129.

10. Golub, K. and M.T. Holtzapple, *Effect of bioreactor mode of operation on mixed-acid fermentations*. 2012, Texas A&M University: College Station, Tex. p. 1 online resource.

11. Gerardi, M.H., *The microbiology of anaerobic digesters*. Wastewater microbiology series. 2003, Hoboken, N.J.: Wiley-Interscience. ix, 177 p.

12. Loescher, M.E., *Volatile fatty acid fermentation of biomass and kinetic modeling using CPDM method*, 1996, Texas A&M University: College Station.

13. Gluszcz, P., et al., *Equilibrium and dynamic investigations of organic acids adsorption onto ion-exchange resins.* Bioprocess and Biosystems Engineering, 2004. **26**(3): p. 185-190.

14. Zagorodni, A.A., *Ion exchange materials: properties and applications*. Vol. xv, . 2007: Elsevier. 496

15. Bhandari, V.M., T. Yonemoto, and V.A. Juvekar, *Investigating the differences in acid separation behaviour on weak base ion exchange resins.* Chemical Engineering Science, 2000. **55**(24): p. 6197-6208.

16. Anasthas, H.M. and V.G. Gaikar, *Adsorption of acetic acid on ion-exchange resins in non-aqueous conditions.* Reactive & Functional Polymers, 2001. **47**(1): p. 23-35.

17. Bhandari, V.M., V.A. Juvekar, and S.R. Patwardhan, *Sorption studies on ion-exchange resins 2. Sorption of weak acids on weak base resins.* Industrial & Engineering Chemistry Research, 1992. **31**(4): p. 1073-1080.

18. Kanazawa, N., et al., *Adsorption equilibrium equation or carboxylic acids on anion-exchange resins in water*. Journal of Colloid and Interface Science, 2001. **238**(1): p. 196-202.

19. Tung, L.A. and C.J. King, *Sorption and extraction of lactic and succinic acids at pH-greater-than-pKa .2. Regeneration and process considerations.* Industrial & Engineering Chemistry Research, 1994. **33**(12): p. 3224-3229.

20. Tung, L.A. and C.J. King, *Sorption and extraction of lactic and succinic acids at pH-greater-than-pKa .1. Factors governing equilibria.* Industrial & Engineering Chemistry Research, 1994. **33**(12): p. 3217-3223.

21. Lv, H.S., et al., *Removal of acetic acid from fuel ethanol using ion-exchange resin.* Energy & Fuels, 2012. **26**(12): p. 7299-7307.

22. Ataei, S.A. and E. Vasheghani-Farahani, *In situ separation of lactic acid from fermentation broth using ion exchange resins.* Journal of Industrial Microbiology & Biotechnology, 2008. **35**(11): p. 1229-1233.

23. Vaccari, G., et al., *Fermentative production of L-lactic acid by Lactobacillus-casei dsm-20011 and product recovery using ion-exchange resins.* Applied Microbiology and Biotechnology, 1993. **40**(1): p. 23-27.

24. Kammerer, J., R. Carle, and D.R. Kammerer, *Adsorption and ion exchange: Basic principles and their application in food processing.* Journal of Agricultural and Food Chemistry, 2011. **59**(1): p. 22-42.

25. Wang Zihao, Z.K., *Kinetics and mass transfer for lactic acid recovered with anion exchange method in fermentation solution.* Biotechnology and bioengineering, 1994. **47**: p. 1-7.

26. Moldes, A.B., J.L. Alonso, and J.C. Parajo, *Recovery of lactic acid from simultaneous saccharification and fermentation media using anion exchange resins.* Bioprocess and Biosystems Engineering, 2003. **25**(6): p. 357-363.

27. Roddick, F.A. and M.L. Britz, *Production of hexanoic acid by free and immobilised cells of Megasphaera elsdenii: Influence of in-situ product removal using ion exchange resin.* Journal of Chemical Technology and Biotechnology, 1997. **69**(3): p. 383-391.

28. Takahashi, S., et al., *Removal of acetic acid from spent sulfite liquor using anion exchange resin for effective xylose fermentation with Pichia stipitis.* Bioresources, 2013. **8**(2): p. 2417-2428.

29. Hua, D.L., et al., *Enhanced vanillin production from ferulic acid using adsorbent resin.* Applied Microbiology and Biotechnology, 2007. **74**(4): p. 783-790.

30. Park, S.W., et al., *Improvement of epothilone B production by in situ removal of ammonium using cation exchange resin in Sorangium cellulosum culture.* Biochemical Engineering Journal, 2007. **37**(3): p. 328-331.

31. Kim, D.H., et al., *Method of extraction and yield-up of tricyclo compounds by adding a solid adsorbent resin as their carrier in fermentation medium*. 2009, Google Patents.

32. Ross, M.K., *Production of acetic acid from waste biomass*. 1998, Texas A&M University. p. xvii, 208 leaves.

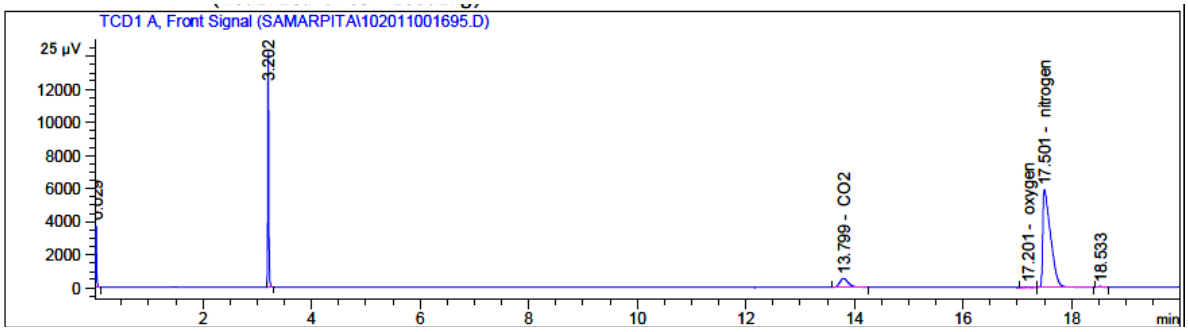
33. NREL, *Biomass analysis technology team laboratory analytical procedure*. 2004: National Renewable Energy Laboratory, Golden, CO.

34. Smith, A.D. and M.T. Holtzapple, *Pilot-scale fermentation and laboratory nutrient studies on mixed-acid fermentation*. 2011, Texas A&M University,: College Station, Tex. p. 1 online resource.

35. Levy, P.F., et al., *Biorefining of biomass to liquid fuels and organic-chemicals.* Enzyme and Microbial Technology, 1981. **3**(3): p. 207-215.

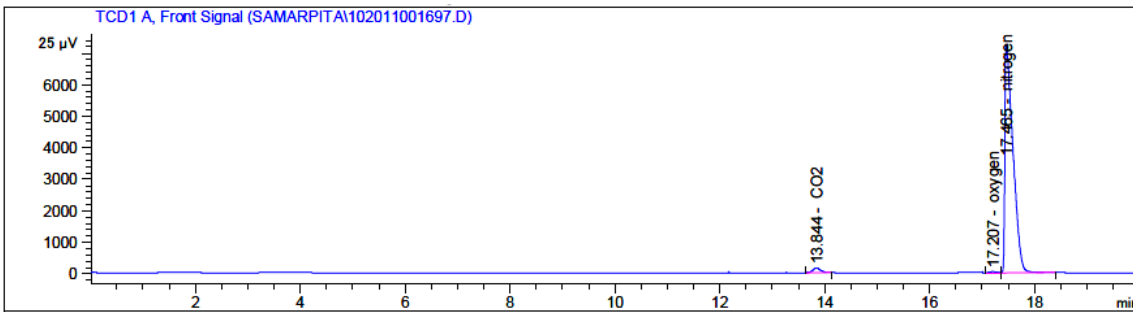
36. Chan, W.N., *Thermophilic anaerobic fermentation of waste biomass for producing acetic acid*. 2002, Texas A&M University, 2002. p. xix, 215 leaves.

APPENDIX A. BIOGAS SAMPLING



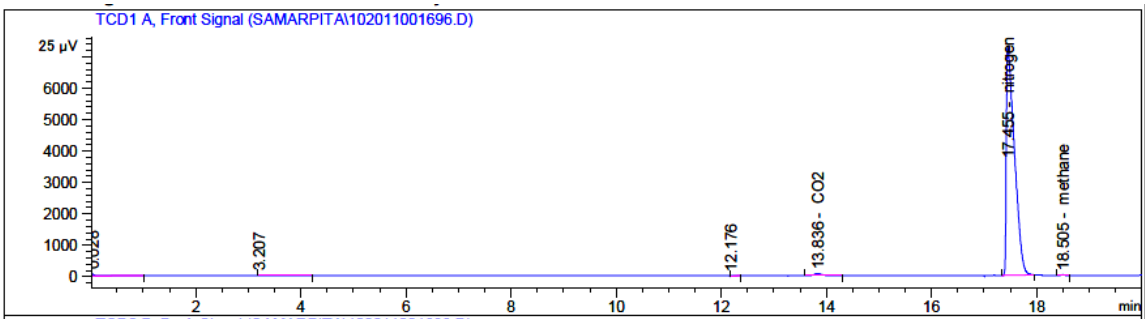
RetTime [min]	Type	Area [25 µV*s]	Amt/Area	Norm %	Grp	Name
13.799	BB	5958.11084	1.13255e-3	8.360687		CO2
17.201	BV	519.35620	1.46711e-3	0.944069		oxygen
17.501	VB S	5.89966e4	1.24074e-3	90.695244		nitrogen
19.086		-	-	-		methane
Totals :				100.000000		

Figure A-1 Biogas sample of α-cellulose fermentor (Day 15).



RetTime [min]	Type	Area [25 µV*s]	Amt/Area	Norm %	Grp	Name
13.844	BB	1503.73450	1.18958e-3	1.820292		CO2
17.207	BV	267.69299	1.63023e-3	0.444081		oxygen
17.465	VB S	7.73659e4	1.24144e-3	97.735627		nitrogen
19.086		-	-	-		methane
Totals :				100.000000		

Figure A-2 Biogas sample of office paper (Day 15).



RetTime [min]	Type	Area [25 µV*s]	Amt/Area	Norm %	Grp	Name
13.836	BB	645.08221	1.25790e-3	0.879382		CO2
17.203	BV	141.85382	1.59055e-3	0.244514		oxygen
17.455	BB S	7.75763e4	1.17611e-3	98.876104		nitrogen
18.505	BB	-	-	-		methane

Figure A-3 Biogas sample of lime pretreated corn stover (Day 15).

APPENDIX B. CARBOXYLIC ACID ANALYSIS

For carboxylic acids analysis, at least 3 mL of liquid is sampled from the fermentor, placed in a 15-mL conical centrifuge tube, and stored in the freezer at –10 °C. When analyzed, the samples were defrosted and vortexed. If the acid concentration is high, it may require further dilution before using the method below.

GC LIQUID SAMPLE PREPARATION

1. Centrifuge the liquid sample for 5 min at 4000 rpm.
2. Pipette 0.5 mL of clear liquid broth into a 2.0-mL microcentrifuge tube.
3. Add 0.5 mL of internal standard 4-methyl-valeric acid (1.162 g/L internal standard, ISTD).
4. Add 0.5 mL of 3-M phosphoric acid to convert all salts to acid form.
5. Cap and vortex the tube.
6. Centrifuge the mixture in a microcentrifuge (8000 × g) for 10 min.
7. Remove the tube and decant the mixture into a glass GC vial and cap. The centrifuged sample in the vial is ready to be analyzed now.
8. If the prepared sample will not be analyzed immediately, it can be frozen. Before GC analysis, make sure to thaw and vortex the sample.

GC OPERATION

1. Before starting the GC, check the gas supply cylinders (compressed hydrogen, compressed helium and compressed air from Praxair Co., Bryan, TX) to insure at

least 200 psig pressure in each gas cylinder. If there is not enough gas, switch cylinders. Make sure to place an order for new ones.

2. Check the solvent and waste bottles on the injection tower. Fill up solvent vials with methanol. Empty the waste vials in designated waste container.
3. Before starting the GC, replace the septum beneath the injection tower.
4. Up to 150 samples can be loaded in the autosampler tray in one analysis batch. Place the samples in the autosampler racks. Include a vial with the volatile acid standard.
5. Check the setting conditions in the method:

    a. Inlet Conditions:

    i. Temperature: 230 °C

    ii. Pressure: 15 psig

    iii. Flow rate: 185 mL/min

    b. Detector conditions:

    i. Temperature: 230 °C

    ii. Air flow rate: 400 mL/min

    iii. H2 flow rate: 40 mL/min

    iv. The (makeup) flow rate: 45 mL/min

    c. Oven conditions:

    i. Initial temperature: 40 °C

    ii. Initial hold time: 2 min

    iii. Ramp rate: 20 °C/min

iv. Final temperature: 200 °C

v. Final hold time: 1 min

d. Total run time per vial: 20 min

6. Start the GC on the computer by selecting the method with the setting conditions mentioned above. Load the sample sequence.
7. For quality control, run the standard mix every 15–25 samples. At the end of the sequence table, set the GC into standby mode to save gas.

APPENDIX C. MOISTURE AND ASH CONTENT ANALYSIS

This procedure was modified from NREL Standard Procedures (2004). If volatile acids are present in sample, lime may be added to retain all acids for more thorough measurement of moisture content (Meysing, 2011). However, when lime is added, the ash content cannot be measured as directed below. In this case, a separate sample must be dried with no lime addition, and subsequently ashed.

1. Record the label and weight of a clean, dry crucible (W1).

2. Place a representative sample of the material (liquid or solid) into the crucible and record the weight (W2).

3. Dry the crucible at 105 °C for 1 day in the drying oven. In a desiccator, allow to cool to room temperature before weighing. Record the dry weight (W3).

4. Ash the crucible at 575 °C for at least 12 h. Remove and allow sample to cool to room temperature in a desiccator. Record the ash weight (W4).

5. The moisture content [1] of the sample is calculated as

$$MC = \frac{W2 - W3}{W2 - W1}$$

6. The ash content (AC) of the sample is calculated as

$$AC = \frac{W4 - W1}{W4 - W1}$$

APPENDIX D. DEOXYGENATED WATER PREPARATION

Deoxygenated water with cysteine hydrochloride and sodium sulfide was used as the liquid medium in all fermentation experiments.

1. Fill a large glass container (≥4 L) with distilled water. Place the container over a hot plate to boil.
2. Boil the distilled water for 10 min.
3. Seal the top of the container and cool to room temperature.
4. Add 0.275 g cysteine hydrochloride and 0.275 g sodium sulfide per liter of boiled water.
5. Stir the solution until both chemicals are completely dissolved and pour into storage tank.

APPENDIX E. BATCH FERMENTATION PROCEDURES

Batch fermentation procedures were initiated in 1-L polypropylene plastic bottles with a rubber stopper capping inserted with a glass tube and two stainless steel pipes that aided mixing of contents of the fermentor. The fermentors were placed in an incubator, set at a temperature of 40˚C, and were monitored every 48 h.

1. *$MgCO_3$ control fermentor monitoring procedure*
    i. Remove the fermentors from the incubator and allow them to cool for 10 min at room temperature.
    ii. Puncture the fermentor septum with a needle and open the valve to release the gases in the fermentor headspace. Record the gas production.
    iii. Remove the fermentor caps and using a nitrogen purge line, carefully remove the residual solids adhered to the stopper and metal bars. Measure and record the pH for each fermentor.
    iv. Use a regular solid centrifuge cap to seal the fermentors. Balance each pair of fermentors on the weighing machine. Pay attention to balance the centrifuge nitrogen. bottles before placing them in the centrifuge.
    v. Centrifuge (4,000 rpm, 25 min) the fermentors to separate the solid and liquid fractions.
    vi. After centrifuging, carefully move the bottles to ensure that the solid and liquid do not remix.

vii. Collect a 1-mL sample of the liquid fraction and store it in a 2-mL centrifuge tube.

viii. Add $MgCO_3$ to the bottles and mix well. Keep adding $MgCO_3$ till the fermentor has reached a near neutral pH.

ix. Add methane inhibitor to each bottle.

x. Mix contents of all bottles thoroughly and purge each fermentor with

xi. Replace fermentor caps and return to incubator.

2. *IR fermentor monitoring procedure*

i. Remove the fermentors from the incubator and allow them to cool for 10 min at room temperature.

ii. Puncture the fermentor septum with a needle and open the valve to release the gases in the fermentor headspace. Record the gas production.

iii. Remove the fermentor caps and using a nitrogen purge line, carefully remove the residual solids adhered to the stopper and metal bars. Measure and record the pH for each fermentor.

iv. Use a regular solid centrifuge cap to seal the fermentors. Balance each pair of fermentors on the weighing machine. Pay attention to balance the centrifuge bottles before placing them in the centrifuge.

v. Centrifuge (4,000 rpm, 25 min) the fermentors to separate the solid and liquid fractions.

vi. After centrifuging, carefully move the bottles to ensure that the solid and liquid do not remix.

vii. Collect a 1-mL sample of the liquid fraction and store it in a 2-mL centrifuge tube.

viii. If the pH of the fermentation broth is less than 6.8, pass the broth through the column until its exit pH is in the range of 6.8–7.2. Draw a sample (1 mL) of the fermentation broth for acid concentration analysis.

ix. If the pH of the fermentor is higher than 7.2 (this may happen during the first few days of the fermentations), bubble CO_2 through into the fermentor.

x. Add methane inhibitor to each bottle.

xi. Mix contents of all bottles thoroughly and purge each fermentor with compressed nitrogen gas.

xii. Replace fermentor caps and return to incubator.

Figure E-1 Experimental setup for IR fermentor monitoring procedure.

Figure E-2 Rolling incubator.

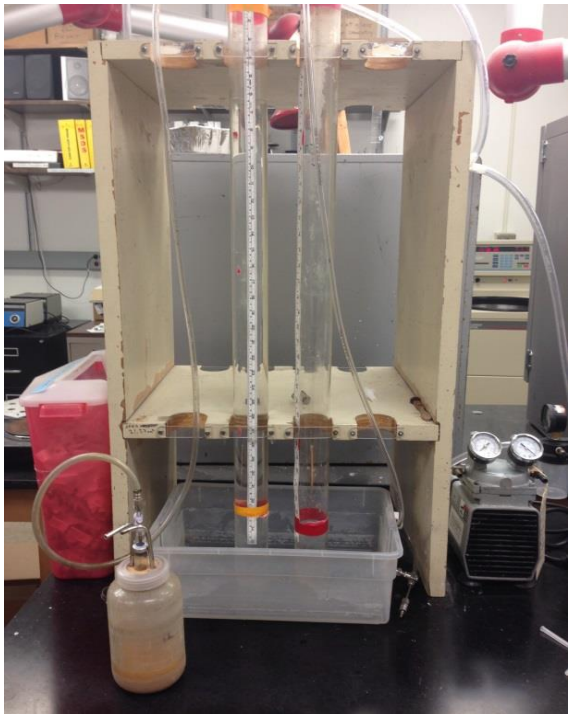
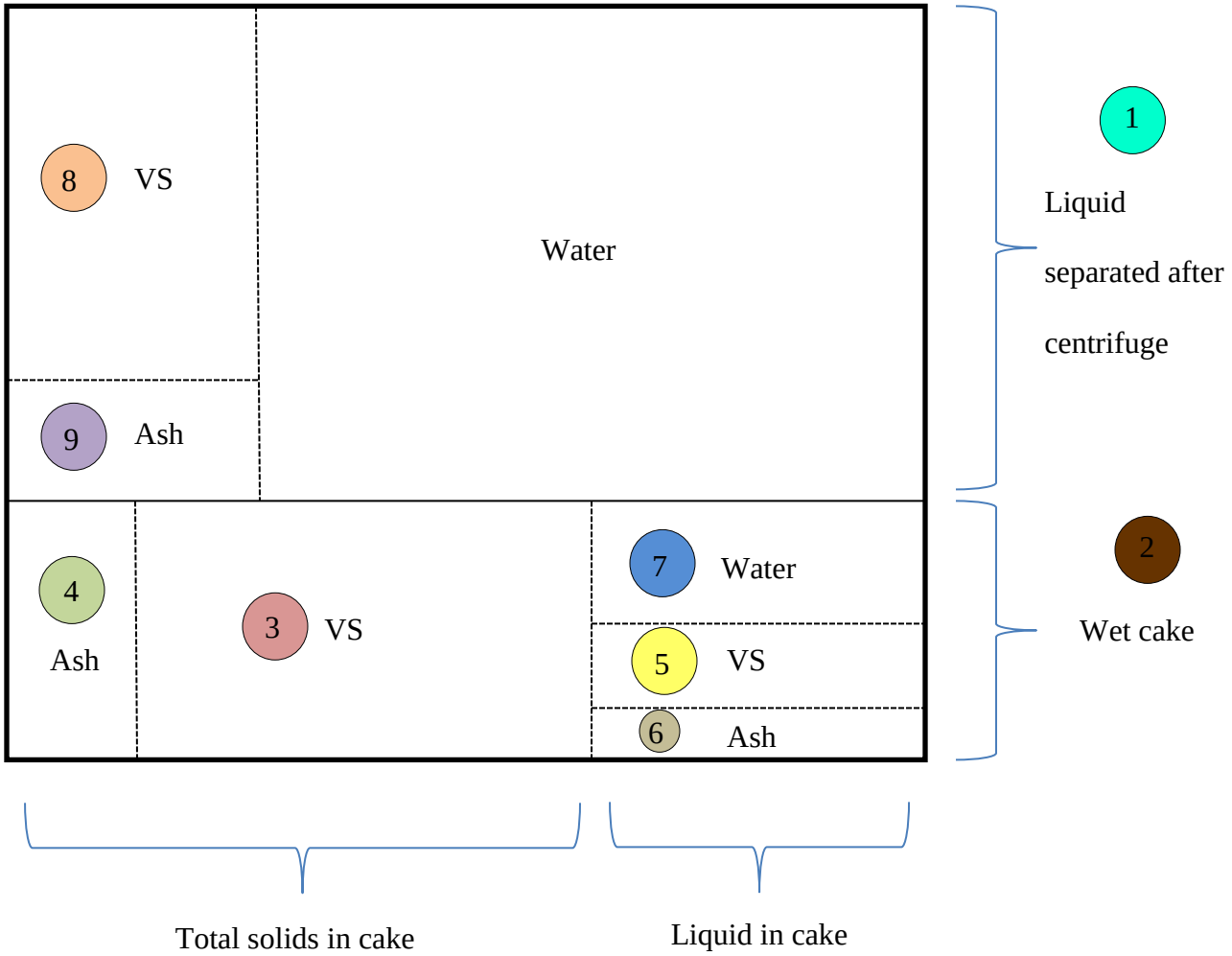


Figure E-3 Biogas measuring cylinder.

APPENDIX F. FERMENTATION PARAMETER CALCULATIONS



Calculated fractions:

1) $\text{Water}_{\text{liquid in cake}} = (\text{Wet cake} \times (1 - \text{TS}_{\text{wet cake}})$

2) $(\text{VS+ Ash+ Water})_{\text{liquid in cake}} = \frac{\text{Water}_{\text{liquid in cake}}}{(1 - \frac{TS_{liquid}}{100})}$

3) $(\text{VS+ Ash})_{\text{wet cake}} = (\text{Cake} \times \text{TS}_{\text{cake separated}})$

4) $\text{Ash}_{\text{wet cake}} = \text{Wet cake} \times \frac{\%\, TS_{cake}}{100} \times \frac{\%\, Ash_{cake}}{100}$

5) $(\text{VS+ Ash})_{\text{liquid in cake}} = (\text{VS+ Ash+ Water})_{\text{liquid in cake}} - \text{Water}_{\text{liquid in cake}}$

6) $(\text{VS+ Ash})_{\text{dry cake solids}} = (\text{VS+ Ash})_{\text{wet cake}} - (\text{VS+ Ash})_{\text{liquid in cake}}$

7) $\text{Ash}_{\text{liquid in cake}} = (\text{VS+ Ash+ Water})_{\text{liquid in cake}} \times \frac{\%\, Ash_{liquid}}{100} \times \frac{\%\, TS_{liquid}}{100}$

8) $\text{VS}_{\text{liquid in cake}} = (\text{VS+ Ash})_{\text{liquid in cake}} - \text{Ash}_{\text{liquid in cake}}$

9) $\text{Ash}_{\text{dry cake solids}} = \text{Ash}_{\text{wet cake}} - \text{Ash}_{\text{liquid in cake}}$

10) $\text{VS}_{\text{dry cake solids}} = (\text{VS+ Ash})_{\text{dry cake solids}} - \text{Ash}_{\text{dry cake solids}}$

11) $(\text{VS+ Ash})_{\text{separated liquid}} = \text{Liquid separated after centrifuge} \times \frac{\%\, TS_{liquid}}{100}$

12) $\text{Ash}_{\text{separated liquid}} = \text{Liquid separated after centrifuge} \times \frac{\%\, TS_{liquid}}{100} \times \frac{\%\, Ash_{liquid}}{100}$

13) $\text{VS}_{\text{separated liquid}} = (\text{VS+ Ash})_{\text{separated liquid}} - \text{Ash}_{\text{separated liquid}}$